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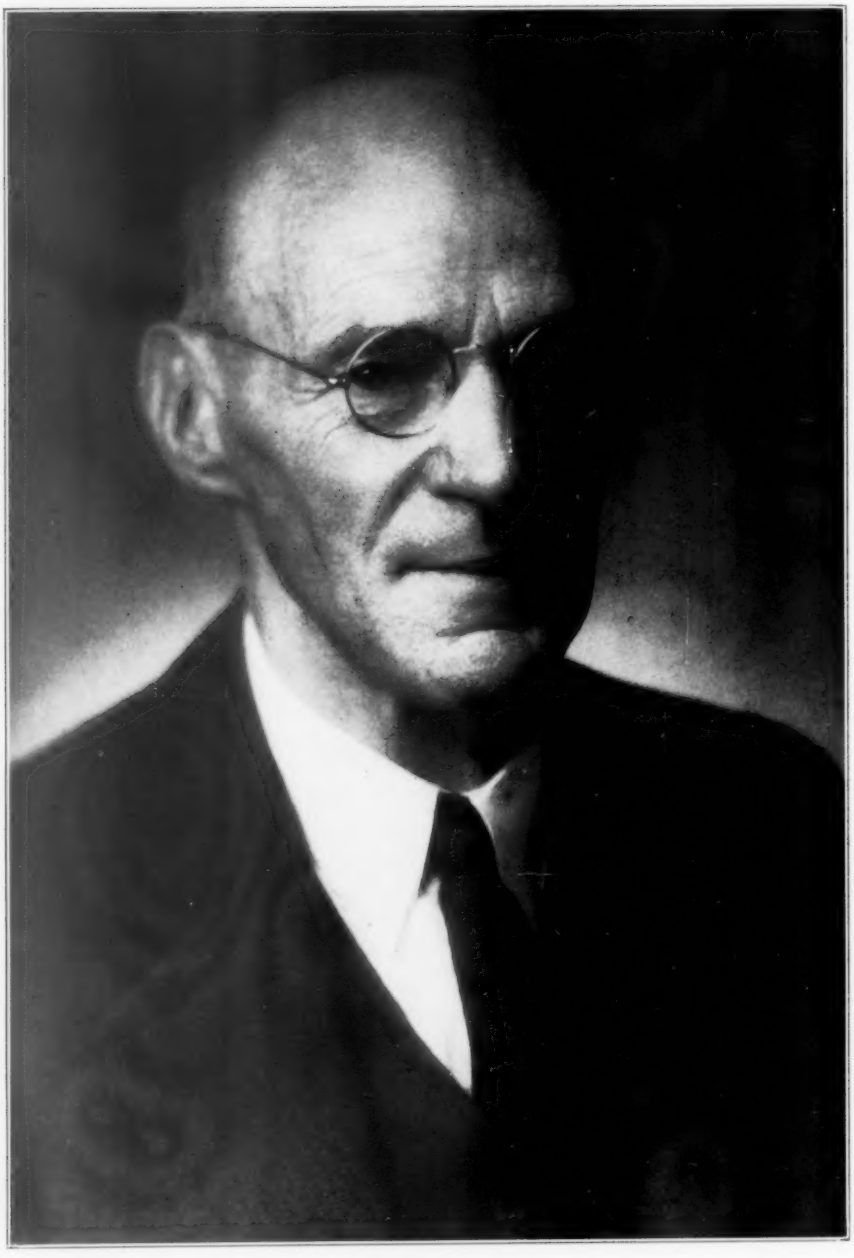

DEANE B. SWINGLE
1879–1944

DEANE B. SWINGLE

1879–1944

H. E. Morris and F. B. Cotner

In the death of Deane B. Swingle at Bozeman, Montana, January 18, 1944, we have lost a distinguished botanist, an eminent teacher, and an excellent administrator.

Deane B. Swingle was born in Wayne County, Pennsylvania, January 6, 1879. He attended country school, was tutored by his mother, and then entered Kansas Agricultural College, obtaining his B.S. degree in 1900. He carried on graduate work at the University of Wisconsin, receiving the M.S. degree in 1901 and the Ph.D. degree in 1931.

From 1901 to 1906 he was connected with the United States Department of Agriculture, working under the direction of Erwin F. Smith.

In August, 1906, he came to Montana State College and organized the work in botany and bacteriology in the Department of Biology. A Department of Botany and Bacteriology was created in 1911, and he remained as head of this department throughout his life. He had charge of the work in botany and bacteriology, specializing in plant pathology, in the Montana Agricultural Experiment Station from 1906 to 1925. In 1931 he was made Dean of the Division of Science of Montana State College. While in this capacity he also served as acting president in President Alfred Atkinson's absence. When A. L. Strand became President, Dr. Swingle was formally named as vice-president and continued to act in this capacity until his death.

Dr. Swingle was a member of the American Association for the Advancement of Science, the Society of American Botanists, American Phytopathological Society (Charter and Life Member), American Society of Plant Taxonomists, Society of American Bacteriologists, and The Montana Horticultural Society (Life Member). He was also a member of Sigma Xi, Phi Kappa Phi, and Phi Sigma, is listed in "Who's Who in America" and in American Men of Science. In the field of botanical sciences he made notable and permanent contributions. His cytological studies concerning the "Formation of the Spores in the Sporangia of *Rhizopus nigricans* and *Phycomyces nitens*" have been quoted and copied by numerous authors of fundamental works in botany. He was the author of three important textbooks: "Textbook of Systematic Botany," "Plant Life," and "General Bacteriology," which have been adopted in many colleges and universities.

Dr. Swingle was loved, respected, and admired by students and faculty alike. His greatest satisfaction was derived from his association with students and in teaching, for he was above everything else a teacher.

His relation to his colleagues was always that of a friend and counselor. He never discredited the opinions of others, even though they differed from his own, but would offer suggestions that would often tend to prove his point. His interest reached far beyond his own particular sphere of endeavor, and

the success of any department was interpreted by him as lending success to his own department, as well as advancing the prestige of Montana State College.

Dr. Swingle believed in people. He found the finest delights of his experience in developing and furthering the interests of others. Although he saw their imperfections he never slackened his efforts to help them to be more nearly the men and women they were capable of being. His work was therefore conceived to be an opportunity for a service that would assure a continuing influence. In this way he developed the kindly, sympathetic, personal philosophy that so charmed his friends and acquaintances and laid the foundation for a true memorial—not in stone or steel, but in the hearts of those who knew him.

To those who knew him intimately the loss of his counsel, his sympathetic understanding, and his friendship will be keenly felt.

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RHIZOME TREATMENTS FOR CONTROLLING BOTRYTIS CROWN ROT IN IRIS[1]

LOUISE DOSDALL

(Accepted for publication February 28, 1944)

Botrytis convoluta was described by Whetzel and Drayton (7) in 1932 as a new species of fungus parasitic on rhizomatous iris. According to these authors the fungus had been intercepted during the ten years previous by United States Department of Agriculture Quarantine Inspectors on iris rhizomes from France, Germany, England, and Holland and had been observed in New York since 1924, in Washington state in 1931, and in Ontario, Canada, in 1927. In 1934 the disease was reported from Minnesota (2), in 1937 from New Jersey (8), in 1938 from British Columbia (1) and Idaho (in correspondence to the author), and in 1939 from Missouri (6); so that it seems to be quite generally distributed at least through the northern part of the United States. A basal gray mold caused by Botrytis species was reported in 1939 (5) as the third most important disease of iris in England, but it is not clear whether the fungus involved is *Botrytis convoluta*. Other references that might be interpreted as pertaining to this species are lacking in the European literature. In 1937 Drayton (3) described the ascigerous stage which he produced in culture and named *Sclerotinia convoluta*.

In the spring of 1934 Botrytis crown rot was very severe in several iris plantings in Minnesota, and in at least three nurseries continued cultivation of iris was threatened because of the destructiveness of the disease and by the great masses of sclerotia and conidia produced as potential inoculum. Rhizome treatments seemed one possible means of preventing spread of the disease and of establishing new plantings free from the disease. In late summer rhizomes from a miscellaneous collection of old varieties of iris were obtained for experimentation from a heavily infected planting that had been observed during the entire growing season of 1934. After the iris had started growth in the spring no further development of the rot was apparent. The fans which had not been killed during the winter grew vigorously and by midsummer there was nothing to indicate presence of the disease except the many missing plants in the rows. As the rhizomes were being cut for replanting, no signs were discovered by which a grower might detect the disease in his stock. With the material available rhizome treatments were planned to obtain as much information as possible concerning the extent to which infection is carried on the rhizomes and the extent to which fungicides are effective in eliminating the disease.

It was common practice among Minnesota iris growers to throw a handful of copper sulphate crystals around the crown of iris plants in the fall to

[1] Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 165-71-1-124, sponsored by the University of Minnesota. Paper No. 2142, the Scientific Journal Series, Minnesota Agricultural Experiment Station.

protect them from rot in general. Mercuric chloride was used to some extent as a rhizome dip to control bacterial soft rot. It was thought that calomel or Semesan might be less injurious, or formaldehyde, if effective, cheaper. These five fungicides were selected and used in the following strengths: mercuric chloride 1:1000, mercuric chloride 1:1000 plus one per cent hydrochloric acid; calomel 1:128 (one ounce to one gallon of water); Semesan 1:400 (0.25 per cent); copper sulphate 1:100; and formalin 1:80, 1:160, and 1:320. For each of the treatments five rhizomes of each of 13 varieties were available. After the rhizomes had been cut into one-fan units and trimmed according to the common nursery practice, each fan-unit with rhizome and the portion 2 to 3 in. above it was treated 5 minutes, drained on newspaper 30 minutes, and wrapped in dry paper until planted

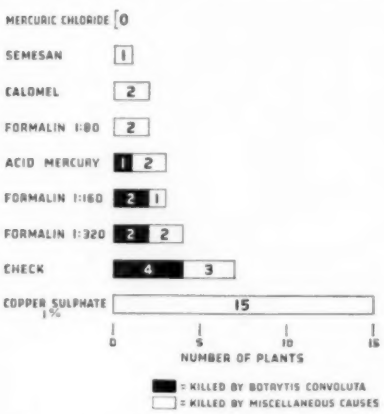

FIG. 1. Results in the spring of 1935 of treating iris rhizomes August 30, 1934, to control Botrytis crown rot. Each bar represents 65 plants belonging to 13 varieties.

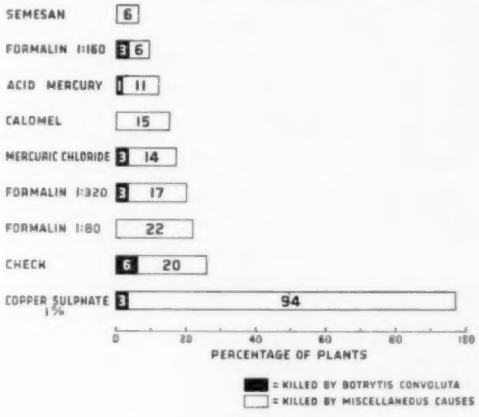

FIG. 2. Results in the spring of 1935 of rhizome treatments August 30, 1934, on the control of Botrytis crown rot in Monsignor, Malvina, and an unknown variety of iris.

five days later. During the winter the plants were covered with a straw mulch. The amount of disease in the plots was observed the following spring.

In the spring of 1935, only 23 (2 per cent) of the 585 plants in the experiment were infected with *Botrytis;* considering the checks alone, 4 plants (6 per cent) out of 65 carried the infection as evidenced by the presence of the fungus (Table 1 and Figs. 1 and 2). This low percentage of rhizomes carrying the disease limited the range within which the treatments exerted their effect. However, 20 per cent of the check plants were lost from miscellaneous causes. This loss was reduced to 17 per cent by formalin 1:320, to 15 per cent by calomel, to 14 per cent by mercuric chloride, to 11 per cent by the acid mercury, and to 6 per cent by both Semesan and the formalin 1:160 (Table 1 and Figs. 1 and 2). Thus these treatments exerted a beneficial effect that is not evident if one considers only plants rotted by *Botrytis* in the spring.

TABLE 1.—*The effects of rhizome treatments on Botrytis crown rot of iris*

Treatments[a]	Check			$CuSO_4$ 1:100			$HgCl_2$ 1:1000			$HgCl_2$ + HCl			Semesan 1:400			Calomel 1:128			HCHO 1:80			HCHO 1:160			HCHO 1:320			Total No. for each variety			Per cent for each variety		
Varieties	M[b]	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H	M	B	H
Miss Maggie	2	0	3	5	0	0	2	0	3	1	0	4	1	0	4	1	0	4	4	0	1	0	0	5	1	0	4	17	0	28	38	0	62
Shakespeare	3	0	2	5	0	0	0	0	5	0	0	5	0	0	5	1	0	4	0	0	5	0	0	5	1	0	4	10	0	35	22	0	78
Edith	0	0	5	3	2	0	0	1	4	0	0	5	0	0	5	0	0	5	1	0	4	0	0	5	2	0	3	6	3	36	13	7	80
Monsignor	2	2	1	5	0	0	0	0	5	1	0	4	0	0	5	0	0	5	1	0	4	1	0	4	1	2	2	11	4	30	25	8	67
Unknown	0	0	5	4	0	1	0	0	5	0	0	5	0	0	5	0	0	5	1	0	4	0	0	5	0	0	5	5	0	40	11	0	89
Rose Unique	2	0	3	5	0	0	2	0	3	1	0	4	0	0	5	1	0	4	2	0	3	0	0	5	4	0	1	17	0	28	38	0	62
Malvina	0	1	4	5	0	0	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	2	3	0	0	5	5	3	37	11	7	82
Gertrude	1	0	4	4	0	1	1	1	3	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	6	1	38	13	2	85
Eugene Sue	2	0	3	5	0	0	3	0	2	3	0	2	1	0	4	4	0	1	1	0	4	1	0	4	1	0	4	21	0	24	47	0	53
Unknown	1	1	3	5	0	0	0	0	5	1	1	3	1	0	4	2	0	3	1	0	4	0	0	5	1	0	4	12	2	31	27	4	69
Unknown	0	0	5	5	0	0	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	5	0	40	11	0	89
Unknown	0	0	5	5	0	0	1	0	4	0	0	5	1	0	4	1	0	4	3	0	2	2	0	3	0	0	5	13	0	32	29	0	71
Unknown	0	0	5	5	0	0	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	0	0	5	5	0	40	11	0	89
Total no. for each treatment	13	4	48	61	2	2	9	2	54	7	1	57	4	0	61	10	0	55	14	0	51	4	2	59	11	2	52	133	13	439			
Per cent for each treatment	20	6	74	94	3	3	14	3	83	11	1	88	6	0	94	15	0	85	22	0	78	6	3	91	17	3	80	0	0	0	23	2	75

[a] Rhizomes of 13 varieties treated August 30 and planted September 4, 1934. Observations May 7, 1935. All plants covered with straw during winter.

[b] Under M are the numbers of plants missing from miscellaneous causes; under B the numbers infected by *Botrytis*, and under H, the numbers remaining healthy.

Originally it was hoped to find some measure of the injury to the plants that might be caused by any of the treatments. Notes were taken in the fall on the condition of the plants just before the straw mulch was applied. At this time a few plants had disappeared. Some of the plants that seemed not to be growing at this time were in good condition the following spring and some that were good in the fall had disappeared in the spring. Unfortunately, from the nature of the disease, considerable time must elapse between rhizome treatment and observations on the effect of treatments in suppressing the disease, in this case from August 30, 1934, to April 1, 1935. During this period it is difficult under Minnesota conditions to follow the progress of the disease and to account for all the things that may happen to the plants. Some of the plants were lost by freezing during the winter. It was discovered later that some of the newly set rhizomes lost during hot dry periods in late summer or early autumn were rotted by *Rhizopus*. Some of the plants that rotted during the winter were inadvertently removed with the straw mulch in the spring. The best criterion of the effectiveness of the treatments therefore seemed to be either the percentage of healthy plants or the combined losses from *Botrytis* and from miscellaneous causes. Where the latter greatly exceeded that in the checks it was assumed to be due to injury from the fungicides; where the number of healthy plants greatly exceeded the number in the checks the benefits were assumed to be due to treatments. Whether all the beneficial effect was due to suppression of the Botrytis rot cannot be stated. The one per cent copper sulphate definitely injured the plants (Fig. 1). The formalin 1:80 and 1:320 plots were not much better than the check plots; and the lowest percentage of plants was lost in the Semesan plots.

Only three varieties, Monsignor, Malvina, and an unknown variety, carried the Botrytis infection in the checks (Table 1). Figure 2 shows the incidence of Botrytis infection in each of the treatments for the 15 plants concerned. This probably gives the better picture of the effect of the fungicides in suppressing the disease even though the number of plants concerned is small.

Experiments of the first year showed that the fungus was carried over from one field to another on the rhizomes to a limited extent. Rhizome treatments at transplanting time gave encouraging results as a control measure. In the three varieties with Botrytis rot in the check plots, mercuric chloride, Semesan, calomel, and formalin 1:80 eliminated the disease; the acid mercury and the weaker formalin solutions reduced the amount; but the copper sulphate 1:100 definitely killed the plants. The mercuries, therefore seemed to offer good prospects for control, formalin seemed deserving of further experimentation as to concentration and length of treatment, and it was apparent that the copper sulphate should be tested at a much reduced strength.

In the various iris plantings observed in the spring of 1934, the heaviest infections of Botrytis crown rot were in those plantings that had been cov-

ered with straw during the winter. To determine whether the rot develops more severely under a covering of straw or when the plants are exposed during the winter, half of 540 plants treated as in the previous experiment were covered with straw and half were not covered. Ten rhizomes of each of six varieties of iris were used for each treatment. One variety, in which no *Botrytis* appeared in either the covered or the not covered check plots, was not included in the summary of results in table 2. Of the 225 plants in the covered series 27 per cent were lost from miscellaneous causes and 2 per cent from *Botrytis,* while in the series where the plants were not covered 28 per cent were lost from miscellaneous causes and 9 per cent from *Botrytis.*

TABLE 2.—*The effects of straw mulch and rhizome treatments on Botrytis crown rot of iris*

Treatments[a]		Plants covered						Plants not covered					
		Killed				Healthy		Killed				Healthy	
		Misc. causes		*Botrytis*				Misc. causes		*Botrytis*			
Fungicide	Strength	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
Check		9	36	2	8	14	56	5	20	11	44	9	36
Copper sulphate	1:100	24	96	0	0	1	4	20	80	3	12	2	8
Mercuric chloride	1:1000	4	16	0	0	21	84	5	20	0	0	20	80
Acid mercury	1:1000	3	12	0	0	22	88	5	20	0	0	20	80
Semesan	1:400	1	4	0	0	24	96	6	24	1	4	18	72
Calomel	1:128	3	12	0	0	22	88	8	32	1	4	16	64
Formalin	1:80	8	32	0	0	17	68	6	24	0	0	19	76
Formalin	1:160	1	4	0	0	24	96	4	16	1	4	20	80
Formalin	1:320	7	28	2	8	16	64	3	12	3	12	19	76
Total		60	27	4	2	161	71	62	27	20	9	143	64

[a] Rhizomes of 5 varieties treated August 30 and planted September 4, 1934. Observations May 7, 1935.

Considering only the check plots, 9 plants in the covered series were lost from miscellaneous causes and 2 from *Botrytis,* a total of 11 of the 25 plants lost; while in the series which was not covered 5 plants were killed by miscellaneous causes and 11 by *Botrytis,* a loss of 16 of the 25 plants. Here again some of the plants killed by *Botrytis* may have been removed inadvertently with the straw mulch so that the effectiveness of the treatments under the two conditions is best depicted by the total loss of plants or by the number of healthy plants.

Figure 3 compares the percentages of plants lost in the two series of each treatment (25 plants). The upper bar of each pair represents the covered plot and the lower bar the plot which was not covered, while the solid portion (white or black) of each bar represents the plants killed by *Botrytis* and the shaded portion those killed by miscellaneous causes. Iris in the copper sulphate plots came through the winter poorly: only one plant escaped

killing in the covered series and three in the series not covered. In general, however, a straw covering reduced the loss of plants in both check and treated plots. The loss attributable to *Botrytis* seemed to be eliminated in most of the treated covered plots and to be greatly reduced in covered check plots. The losses resulting from miscellaneous causes were slightly or greatly reduced by straw covering plus certain of the treatments (mercuries and formalin at 1:160) but were not reduced with straw covering alone or with straw covering plus copper sulphate or formalin at 1:80 and at 1:320. Rhizome treatment alone, without straw covering, always reduced or eliminated the loss from *Botrytis* but seldom reduced the losses from miscellaneous causes.

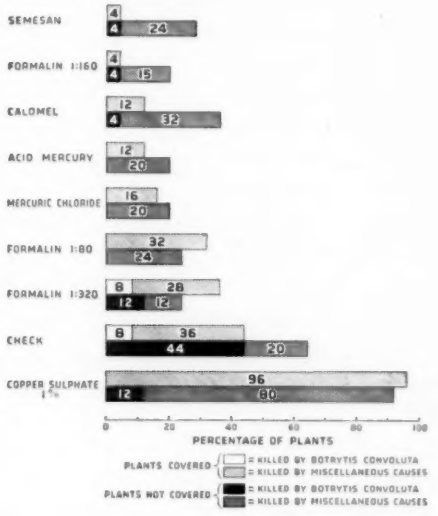

FIG. 3. Comparison of the amount of Botrytis crown rot developed from treated iris rhizomes with and without a straw covering during the winter 1934–1935. Each bar represents 25 plants belonging to 5 varieties.

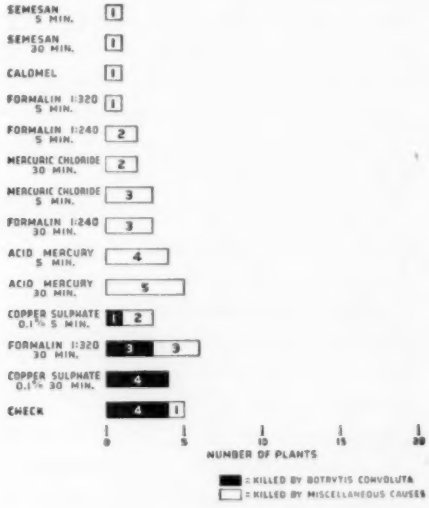

FIG. 4. Results in the spring of 1936 of rhizome treatments October 1, 1935, on the control of Botrytis crown rot. Variety Labor.

Thus under the conditions of this experiment, fewer plants were lost from Botrytis crown rot when the plants were covered with a straw mulch than when they were left exposed during the winter.

In the fall of 1935 it was possible to obtain from a private nursery a quantity of rhizomes of the variety Labor. These plants had been divided and set out in the late summer of 1933. In the spring of 1934 there had been considerable loss from Botrytis rot although exact counts on the percentage of plants infected were not made. Frequently several adjacent plants in a row were rotted by *Botrytis*, then for a distance all the plants were healthy. In some cases an entire row had been killed. The spotted distribution of the diseased plants in this field particularly had suggested that the infection was carried to the field on the rhizomes. This planting was reported to have very little Botrytis rot in the spring of 1935.

When the rhizomes of these plants were cut into one-fan units for replanting they were perfectly sound and there was no striking evidence of disease. The field evidence seemed to be that the disease had disappeared from the planting. The outer leaves, however, were dry on almost all the fans and at the base of the leaves there was a leathery dry rot similar to that from which *Botrytis convoluta* was isolated in the fall of 1934. No tissue cultures were made from this material, however. These outer leaves did not separate and leave a clean scar as do normally senescent leaves; they tore irregularly, leaving fragments of dead leaf tissue attached to the rhizomes.

The same method of rhizome treatment was followed as in 1934. The strength of the copper sulphate solution was reduced to 1:1000, the formalin 1:80 was omitted and a 1:240 was substituted for the 1:160. For all treatments except the calomel, 5-minute and 30-minute immersion periods were tested. Twenty rhizomes were used for each treatment. The rhizomes were treated October 1, 1935, and planted October 2, in two blocks with the 14 treatments randomized in each. Final notes on the number of plants killed by miscellaneous causes, killed by *Botrytis,* and infected by *Botrytis* but not killed were recorded May 13, 1936. All of the plants were covered with straw during the winter. More accurate counts than in the previous year of the number of plants killed by *Botrytis* were obtained by examining each individual plant as the straw was removed from it.

The results are summarized in figure 4. Each bar represents the number of plants lost, the clear portion those lost from miscellaneous causes and the black portion those from *Botrytis.* One plant in the 30-minute copper sulphate plot was infected with *Botrytis* but was not killed. In 1937 this same plant developed no signs of the disease. In all other cases the plants showing signs of *Botrytis* were dead in the spring of 1936. No *Botrytis* appeared in any of the plots except the checks, the two copper sulphate and the 30-minute formalin 1:320 treatments. The best stands were obtained with the two Semesan, the calomel, and the five-minute formalin 1:320 treatments. With the 30-minute acid mercury and the 30-minute formalin treatments as many plants were lost as in the check plots. The significance of these results was evaluated by calculating the analysis of variance for the number of living plants in each plot since the majority of the plots contained no plants with signs of Botrytis infection. With regard to stand there were no statistically significant differences in the response of the rhizomes to the various treatments. However, from the standpoint of elimination of the disease the results were consistent with those of the previous year, namely, the mercury treatments gave good promise of adequate control, the formalin fair promise that the proper combination of strength of solution and period of immersion might be worked out, and the copper sulphate seemed unsatisfactory.

In the summer of 1937 rhizomes of the plants set out in 1934 were available for experimentation. This block of plants had been under continuous observation since planted. In the spring of 1937 a high percentage of

plants was diseased as a result of inoculations with macroconidia of *Botrytis convoluta* the previous November. Moreover, Botrytis rot resulting from natural infection was extremely heavy in the spring of 1937. This planting consequently offered the most uniformly infected lot of plants that had been available for study. During April and May each clump of iris infected with *Botrytis convoluta* was marked with a stake. The rhizomes from these plants were used in the late summer for further rhizome treatment experiments.

In spite of the heavy infection of Botrytis rot in the spring, the new rhizomes in late summer showed no signs of disease. In some varieties the soil close to the roots contained many black sclerotia of *Botrytis convoluta* which sometimes adhered very closely in the crevices between the roots and rhizomes. These sclerotia were particularly abundant among the roots of the variety Miss Maggie.

The same general method of treatment was followed as in the two previous years. The strength of the formalin solutions was changed to 1:200 and 1:400. A dry and a wet sulphur treatment were added to the list. In the dry treatment the rhizomes were shaken in a paper bag with a small quantity of dusting sulphur, in the wet treatment they were dipped in a suspension of wettable sulphur containing one pound sulphur in a gallon of water. Mercuric chloride and calomel were used in combination as well as singly. A yellow mercury oxide treatment was included also. Both the calomel and the yellow oxide were used simply in water suspensions and combined with gum arabic.

For making the gum arabic suspensions the recommendations of Glasgow (4) for the use of calomel in treating cabbage seed were followed. One part of gum arabic was ground as finely as possible in a porcelain mortar, a small amount of water added and ten parts of the mercury powder ground in to make a smooth paste. Finally enough water was added to give a proportion of one ounce of calomel or yellow oxide to one gallon of water. The gum held the calomel in suspension for several hours but in 24 hours it had settled out. The yellow oxide and the gum combination was not much more stable than the yellow oxide alone.

Twenty rhizomes of each of five varieties received each treatment on August 27, 1937, and were planted the following day in 2 randomized blocks in soil which had not grown iris previously. All the plots were covered with straw during the winter. Final counts on the number of plants infected with *Botrytis,* the number of plants killed by miscellaneous causes, and the number of healthy plants were recorded May 23, 1938. From these data the response of the iris rhizomes to the various treatments was again studied by calculating the analysis of variance for the number of living plants free from Botrytis infection.

The results are summarized in table 3. Examining the mean numbers of healthy plants for all the varieties, it is seen at once that all mercury treatments except the 10-minute Semesan gave highly significant superior

TABLE 3.—*Results of rhizome treatments to control Botrytis crown rot. Rhizomes treated August 27 and planted August 28, 1937; observations May 23, 1938. All plants were covered with straw during the winter*

Treatment			Mean No. healthy plants per plot of 10 plants					
Fungicide	Strength	Time in minutes	All varieties	Miss Maggie	Shakespeare	Edith	Monsignor	Columbia
Calomel	1:128	10	**9.7	**9.5	10.0	10.0	*9.5	9.5
Calomel and gum arabic	1:128	10	**9.5	**9.5	10.0	9.0	*9.5	9.5
Mercuric chloride and calomel	1:1000	30	**9.4	**9.0	9.5	9.0	*10.0	9.5
Yellow mercury oxide and gum arabic	1:128	10	**9.4	**8.5	9.0	9.0	*10.0	10.0
Yellow mercury oxide	1:128	10	**9.3	**9.0	9.5	9.0	*10.0	9.5
Mercuric chloride and calomel	1:1000	10	**9.2	**9.5	9.5	8.5	*10.0	8.5
Mercuric chloride	1:1000	30	**9.1	*8.0	9.5	9.0	*9.5	9.5
Semesan	1:400	30	**9.0	7.0	8.5	10.0	*9.5	10.5
Mercuric chloride	1:1000	10	**8.7	*7.5	9.5	8.5	*9.0	9.0
Acid mercury	1:1000	30	**8.6	7.0	8.0	9.5	*9.5	9.0
Acid mercury	1:1000	10	**8.5	6.5	9.0	9.5	*8.5	9.0
Formalin	1:200	30	*7.8	3.5	8.0	9.0	*9.5	9.0
Formalin	1:400	10	7.3	5.5	8.0	8.5	5.5	9.0
Semesan	1:400	10	6.7	4.5	6.0	8.5	7.0	7.5
Sulphur—wettable	1:8	10	6.7	5.0	7.0	8.5	5.0	8.0
Formalin	1:400	30	6.6	2.0	9.0	8.0	6.5	7.5
Formalin	1:200	10	6.5	4.5	6.5	8.5	5.5	7.5
Sulphur—dusting			6.4	4.0	6.0	7.0	5.5	9.5
Check			6.5	4.0	8.0	8.0	5.0	7.5
Mean No. healthy plants per plot			8.15	6.53	8.45	8.78	8.13	8.87

	at 5 per cent level	at 1 per cent level
Difference necessary for significance between treatments	1.1	1.5
Difference necessary for significance between treatments within any variety	3.45	5.71
Difference necessary for significance between varieties	0.57	0.94

* = Significant difference between treatments at the 5 per cent level compared with the check.

** = Significant difference between treatments at the 1 per cent level compared with the check.

stands when compared with the checks and that the 30-minute formalin 1:200 was significantly superior to the check when compared at the five per cent level. The three other formalin treatments and the sulphur treatments were not significantly superior to the checks. There were no signifi-

cant differences between the effective mercury treatments except between the calomel treatment with the highest and the 10-minute acid mercury with the lowest mean. Gum arabic added nothing to the effectiveness of the calomel or yellow oxide of mercury, nor was the combination of calomel and mercuric chloride more effective than either fungicide alone.

Considering the mean numbers of plants for each variety, there is a significant difference in the response of the variety Miss Maggie to the treatments in comparison with the rest of the varieties even at the one per cent level. There is also a significant difference between the response of the variety Monsignor and varieties Columbia and Edith at the five per cent level. The Botrytis infection in the check plots was 50 per cent in the variety Miss Maggie, 15 per cent in Shakespeare, 15 per cent in Edith, 50 per cent in Monsignor, and 25 per cent in Columbia. The loss of plants from miscellaneous causes in the check plots was 10 per cent in Miss Maggie, 5 per cent in Shakespeare, 5 per cent in Edith, and none in Monsignor and Columbia. Thus, the varieties Miss Maggie and Monsignor carried considerably more Botrytis infection on the rhizomes than did the others and in the variety Miss Maggie an additional 10 per cent of the plants were lost from miscellaneous causes.

Examining the response of the individual varieties to the various treatments it is seen that significant differences between the checks and the treatments were obtained in the two varieties Miss Maggie and Monsignor, with the most marked response in the former. In the latter variety the acid mercury treatments and the 30-minute Semesan treatment as well as the 30-minute formalin 1 : 200 gave no significant differences in comparison with the checks, while the mercuric chloride treatments were significantly superior to the checks only at the five per cent level. In another experiment, however, with this same variety, where 40 rhizomes were used for each treatment, both the mercuric chloride treatments were significantly superior to the checks at the one per cent level.

In the tests of the two previous years there seemed to be considerable difference in the number of fans per plant in the different plots. It was thought that this might be an indication of a stimulatory or injurious response of the rhizomes to the various treatments or a varietal characteristic. A record was accordingly kept of the number of fans produced by each plant and the analysis of variance calculated for this character. No statistically significant differences were found for either the treatments or the varieties.

Since the plot from which the iris were taken in the late summer of 1937 had been so heavily infested with *Botrytis convoluta* in the spring, it offered a good opportunity to test the effect of the fungicides against soil-borne infection. A series of treated rhizomes paralleling the series planted in new soil was planted in the infested soil. After the iris had been removed from the field the soil was plowed, disced and harrowed. In replanting, the new rows were laid out in as nearly the same position as the former rows as was

possible. Sufficient space was available for six blocks of treatments similar to those planted in the new soil. There were rhizomes enough of three of the varieties used for the treatments in new soil, namely varieties Shakespeare, Edith, and Monsignor, to plant one series of 10 rhizomes for each treatment in the infested soil, or 190 rhizomes of each variety. The other three blocks were filled with three different varieties. The iris were dug August 31, the rhizomes treated September 2 and replanted September 3,

TABLE 4.—*Effect of rhizome treatment on Botrytis crown rot in iris planted in new and in infested soil, winter of 1937–1938*

Treatment		New soil (per cent plants)[a]			Infested soil (per cent plants)		
Fungicide	Time	Healthy	Killed by misc. causes	Infected by Botrytis	Healthy	Killed by misc. causes	Infected by Botrytis
Calomel	10	97	0	3	70	0	30
Calomel and gum arabic	10	95	0	5	74	3	23
Mercuric chloride and calomel	30	94	2	4	67	2	31
Yellow mercury oxide and gum	10	94	2	4	72	3	25
Yellow mercury oxide	10	93	1	6	73	0	27
Mercuric chloride and calomel	10	92	2	6	68	2	30
Mercuric chloride	30	91	2	7	57	2	41
Semesan	30	90	0	10	50	10	40
Mercuric chloride	10	87	6	7	59	3	38
Acid mercury	30	86	3	11	67	3	30
Acid mercury	10	85	3	12	38	8	54
Formalin 1: 200	30	78	4	18	54	8	38
Formalin 1: 400	10	74	3	23	45	0	55
Sulphur—wettable	10	67	4	29	50	3	47
Semesan	10	67	5	28	43	7	50
Formalin 1: 400	30	66	5	29	32	7	61
Formalin 1: 200	10	65	3	32	54	8	38
Sulphur—dusting	.....	64	8	28	38	12	50
Check	.....	65	4	31	32	15	53
Average		81	3	16	55	5	40
Average of the 3 varieties in both plots		84	3	13	53	7	40

[a] Percentages in the new soil columns are based on 100 plants. Percentages in the infested soil columns are based on 60 plants.

1937. On March 21, 1938, while the ground was still frozen, some of the straw covering the plants was lifted and *Botrytis convoluta* conidia were found on several plants. Final observations on the amount of the disease in the field were made May 24, 1938.

A comparison of the results obtained from this planting of treated rhizomes in infested soil with those obtained from planting treated rhizomes in clean soil is given in table 4. Percentages are tabulated of the plants living in spring but not infected with *Botrytis*, of the plants killed by miscella-

neous causes but not attributable to *Botrytis* directly, and of the plants showing definite signs of infection by *Botrytis*. The fungicides are listed serially in a descending scale according to the percentage of healthy plants in the new soil. According to this scale, the mercury treatments fall at the upper end and the formalin, sulphur, and check treatments at the lower. The percentages of plants killed by miscellaneous causes and the percentages of plants infected by *Botrytis* in general fall in the reverse order. This would indicate again that some of the unaccounted for loss of plants was actually due to factors controlled by the treatments, in this experiment perhaps most of it. The percentages of healthy plants run in almost the same sequence in the two types of soil but they are considerably lower in the new soil. The percentage of plants infected by *Botrytis* is from two to ten times as great for plots in infested soil as for the corresponding ones in new soil. In general, the mercuries tend to suppress Botrytis rot, but rhizome treatment is of little practical importance when the rhizomes are planted in heavily infested soil.

This relative effectiveness of rhizome treatment for three varieties in infested soil and in new soil is represented more strikingly in figure 5. Diseased and missing plants in new soil are represented by white bars and the plants diseased and missing in the infested soil by black bars, the solid portion of the bar in each case representing the percentage of plants infected by *Botrytis* and the shaded portion those lost by miscellaneous causes. The percentage in each white bar is thus based on 60 plants and the percentage in each black bar on 30 plants. For ease of comparison the white bars were superimposed on the black bars. Treatments are ineffective when the rhizomes are planted in infested soil except that there is a tendency for the fungicides effective in new soil to suppress the infection in infested soil. Calomel and gum arabic is considerably more effective in suppressing Botrytis crown rot in infested soil than calomel alone is. In all conclusions drawn from this experiment, however, it must be borne in mind that the pathogen was probably not distributed evenly through the soil in the infested plot since all of the iris in the previous planting were not infected. The plowing, discing and harrowing between plantings helped to distribute whatever sclerotia, spores and mycelium were in the soil but probably not so evenly as would be desired for a careful analysis. To compensate for this heterogeneity a large number of replications would have been desirable.

Finally, an attempt was made to determine whether rhizomes from plants visibly infected with *Botrytis convoluta* in the spring carried the infection more often than did those from plants in the same field showing no such signs of spring infection. In the spring of 1937 each clump of iris on which were found macroconidia, sclerotia, or the characteristic tan felty rot of the fungus was marked with a stake. In the late summer the rhizomes from these plants were harvested separately. For the variety Miss Maggie 400 rhizomes from the plants with signs of infection and 400 from plants with no signs of infection were subjected to the same series of treatments as those

used in the other experiments of this same year and then planted in new soil. Of these 800 rhizomes, 31 per cent of those from plants showing visible signs of *Botrytis* in the spring of 1937 were infected with *Botrytis* in the spring of 1938 and 26 per cent from plants with no signs of infection in the spring of 1937 were infected in 1938. Considering only the untreated rhizomes (40 in each case) 50 per cent from the plants with signs of infection in 1937 were visibly infected in 1938 and 40 per cent from the plants not visibly infected in 1937 were visibly infected in 1938.

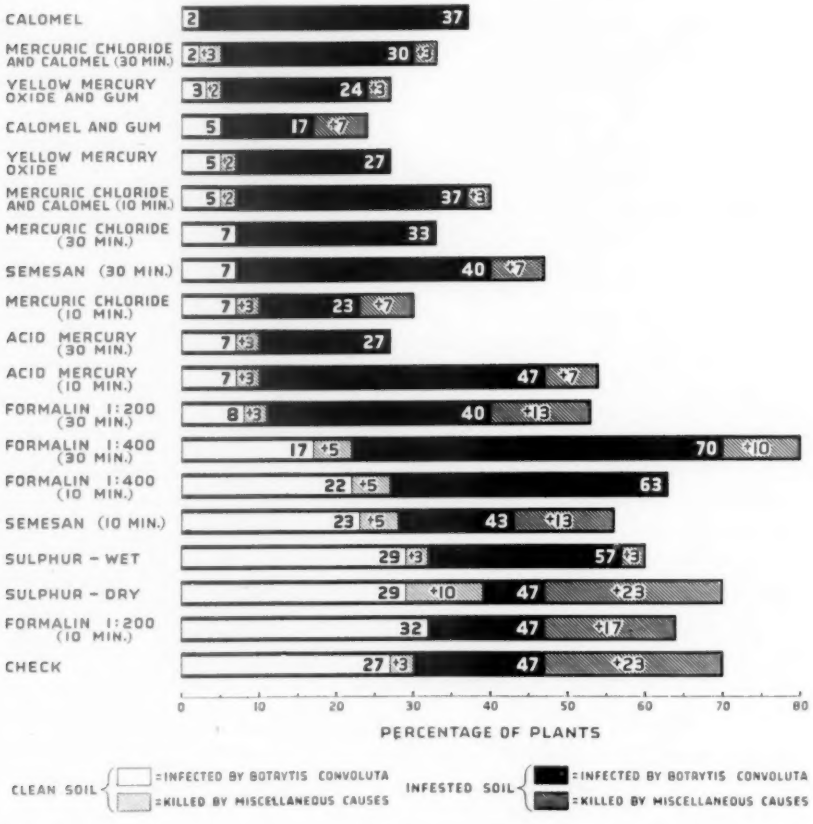

FIG. 5. Comparison of the effect of planting treated rhizomes in infested and in noninfested soil.

An analysis of variance was made for the number of healthy plants in the series of treated plots. There was no statistically significant difference between the number of healthy plants obtained from the rhizomes from infected plants and those from plants not infected. An analysis was also made comparing the number of plants visibly infected with *Botrytis* in the spring of 1938 from rhizomes of plants bearing visible signs of the fungus in the spring of 1937 and from plants bearing no such signs. The differences were not statistically significant. The conclusion is that when rhizomes are

taken from a planting of iris heavily infested with *Botrytis convoluta,* apparent freedom from disease in the spring is no assurance that the rhizomes will not carry the pathogen to a new planting.

In the late summer of 1939 the mercury treatments were tested once more, since these had given the most promising results in 1934, 1935, and 1937. Calomel, yellow mercury oxide, mercuric chloride, and Semesan were used. The first two fungicides were used again at the rate of one ounce to one gallon of water (1:128) both with and without the gum arabic. In the gum combinations twice the amount of gum, 20 per cent the weight of the chemical, used in 1937 was tested in an attempt to make the suspensions more stable. In these proportions the yellow oxide remained in suspension somewhat better than the calomel. This was exactly the reverse of the

TABLE 5.—*The effects of rhizome treatments with mercury fungicides on Botrytis crown rot in the iris variety Edith*

Treatment[a]			Mean No. healthy plants (20 replicates)	Percentage of plants		
Fungicide	Strength	Minutes		Healthy	Killed by misc. causes	Infected by *Botrytis*
Semesan	1:200	30	4.80	96	1	3
Calomel and mercuric chloride	1:128 1:500	30	4.75	95	2	3
Calomel	1:128	Dip	4.70	94	1	5
Mercuric chloride	1:500	30	4.60	92	3	5
Yellow mercury oxide	1:128	Dip	4.55	91	2	7
Calomel and gum	1:128	Dip	4.50	90	4	6
Yellow oxide and gum	.....	Dip	4.45	89	4	7
Check	.....	.....	4.05	81	5	14

Difference between means necessary for significance at the
5 per cent level = 0.036
1 per cent level = 0.047

[a] Rhizomes treated and planted September 14, 1939. Observations May 21, 1940.

experience in the previous trials. Mercuric chloride and Semesan were used at twice the strength used before and with only the 30-minute soak for each.

Rhizomes of only one variety of iris, Edith, were used in this experiment. The plants from which these were taken had been in the field two years. In the spring of 1938, 27 per cent were infected with *Botrytis convoluta* and in the spring of 1939, 18 per cent. The plants were dug September 6 as they occurred in the field without any attempt to select only those plants which bore visible signs of the fungus the preceding spring. At cutting time there was no decay in the rhizomes but in many the outer leaves were dry and tore irregularly from the rhizome. One hundred one-fan units were used for each treatment. The rhizomes were treated in the field on September 14 and planted immediately after being taken from the solutions.

One hundred rhizomes were treated together, then separated into lots of five, and planted in 20 different blocks. The eight treatments were randomized within each block. Thus there were 20 replications for each treat-

ment. Final records of the number of living plants free from visible signs of Botrytis infection, the number of plants dead or missing from causes not attributable to *Botrytis* directly, and the number of plants visibly infected or killed by *Botrytis* were made May 21, 1940.

To test the effectiveness of the various treatments an analysis of variance (Table 5) was made for the number of living plants in the spring of 1940 free from visible signs of Botrytis infection. In the check plots 14 per cent of the plants were infected with *Botrytis convoluta* and 5 per cent were killed by miscellaneous causes. Thus the rhizomes carried a relatively low amount of infection. From a comparison of the mean number of healthy plants per plot all of the treated plots produced a stand significantly better than the check and the individual treatments can be rated for effectiveness in the order of their means. Gum arabic decreased the effectiveness of both the calomel and the yellow oxide of mercury. The calomel and mercuric chloride treatment was statistically superior to the calomel alone but the one per cent increase in stand would scarcely be of practical significance. In this same variety, with rhizomes carrying the same amount of infection there were no significant differences between the mercury treatments in the 1937 experiment when only 20 rhizomes were used for each treatment.

DISCUSSION

Along with the experiments on treating rhizomes to control Botrytis crown rot other studies were undertaken to learn more of the nature of the disease, its development from year to year under known field conditions and its destructiveness over a period of years. Under the climatic conditions which prevail in Minnesota Botrytis crown rot is primarily a disease of the dormant plants. The fungus itself, in relation to the iris plant, appears to be dormant or inactive during the summer. In September or October, in some years, depending upon weather conditions, it may be found sporulating inconspicuously at the base of the outer leaves or old flower stalks. In other years there are no signs of the disease in fall. Sclerotia too have been found forming in October on the cut surfaces of newly divided rhizomes. In general, however, at the time the iris are covered with the usual straw mulch in October or November, or when the natural snow covering falls, the plants appear in excellent condition. At the time the covering is removed in March or April many plants may be rotted and quantities of sclerotia and conidia found on the plants.

The period of maximum activity of the fungus varies greatly from year to year depending apparently on weather conditions. In some years practically all the damage is done under the straw or snow covering. If the weather is warm and dry in April so that the iris start growing rapidly, no further damage is done. If, on the other hand, it is cool for several weeks, or months even, the rot will continue to develop and sclerotia and conidia will be formed in great abundance. Thus under Minnesota conditions the activity of the fungus in the plant may cease suddenly in March or it may continue into June.

It is only during this period in spring that the disease is easily detected in an iris planting. Yet it is mostly overlooked, because the plants which fail to develop are considered by the average grower to have been lost through winter injury. The mass of conidia at the base of the plant is such a neutral grayish brown that it is difficult to distinguish from the surrounding soil. Then, too, most of the evidence of the disease is removed with the covering or in cleaning away the dead leaves. When iris are divided in late summer there are no easily detected signs of the disease, so that the necessity of treatment at this stage is not recognized. The fact that the causal agent is carried on rhizomes which show no signs of infection and even on rhizomes which come from plants showing no signs of infection in spring is of importance to the individual purchaser of iris and to the nursery grower.

The severity of the disease varies greatly from year to year even in the same planting. A high percentage of loss one year may be followed the next with only a trace of the disease in the same field. However, in every planting in which the disease has been found, it has persisted from year to year unless special measures were taken to eliminate it. The apparent absence of the disease for a year or two, or even for several years, gives the impression that the disease is not serious and tends to disappear of itself and also leads the grower to minimize the need of control measures when they will be most effective.

The ineffectiveness of the rhizome treatments in infested soil makes evident the necessity of an adequate system of crop rotation combined with effective rhizome treatments. That these measures have given practical results has been demonstrated by nursery growers. One grower who has very carefully cleaned up his stock has obtained circumstantial evidence that the disease is more widespread in the United States than survey reports would lead one to believe, since rhizomes received from points extending to the east coast, the west coast and as far south as Tennessee have developed the disease when planted in his nursery without rhizome treatment while his own stock remained free from the disease. He now has made it a regular practice to treat all stock brought into his nursery from the outside.

Rhizome treatment has proved to be a more complex problem than simply finding a satisfactory fungicide and brings up a number of questions which need more careful attention. There seems to be a marked difference in the frequency with which rhizomes of some varieties carry the infection, although on individual plants the disease seems to be just as destructive in one variety as in another. Is this an indication of a type of resistance and how and where is the fungus carried on the rhizomes? Is rhizome treatment more necessary for some varieties than for others? Also, different varieties respond differently to the same treatments. The marked failure of the variety Miss Maggie to respond to the 30-minute Semesan and the acid mercury treatments in the 1937 experiments brings out the necessity for the study of the behavior of fungicides in relation to specific varieties. The

relationships between various types of winter covering and the development of Botrytis crown rot as well as the protection these coverings provide the treated rhizomes need further study. The four most destructive occurrences of the disease that have been observed were all in areas which had been covered with several feet of snow during the winter.

While the mercury treatments in general gave good results, the wholly unsatisfactory tests with copper sulphate by no means eliminate the possible effectiveness of other copper preparations. Systematic experimentation with formaldehyde solutions might lead to adequate procedures, although the nicety of balance between the strength of solution, the length of treatment and the variety of iris treated will evidently be finer than with the mercuries. In addition to the results of the experiments recorded, the experience in one nursery in the first years of the study seems to indicate that formaldehyde even in dilutions too weak to be effective burned the foliage of some of the varieties to such an extent that the plants did not become established before cold weather set in. The possibilities of many other compounds are not to be overlooked.

The general conclusions to be drawn from this study are that *Botrytis convoluta* has high potentialities as a destructive agent in iris plantings. Because the greatest damage is done during the dormant period of the host and the signs of the disease are inconspicuous, it is usually overlooked and the injury is dismissed as winter killing. Even though the plants are apparently unaffected during the growing season and there are no signs of decay on the rhizomes at transplanting time, rhizomes from an infested planting nevertheless carry the infection which, however, does not make itself manifest until the following spring. With the extensive interchange of rhizomes that takes place the implication is that the disease must occur practically wherever iris are grown. The disease may cause greater damage in some regions than in others but wherever many iris are lost by so-called winter killing a more careful study should be made to determine how much of this is really due to *Botrytis convoluta* and how much can be eliminated by rhizome treatment.

SUMMARY

During four years 5330 iris rhizomes belonging to 14 varieties were treated in various ways to determine to what extent *Botrytis convoluta* is carried on the rhizomes and whether the fungus can be eliminated by rhizome treatment.

Without any outward signs of disease, rhizomes from an infested planting carry the pathogen to a varying degree depending upon the season and apparently upon the variety.

With rhizomes from a heavily infested planting, no significant differences were found in the number of rhizomes carrying the infection, whether from plants bearing conidia, sclerotia or typical rot in the spring or from plants with no such signs of the disease.

The disease is easily overlooked and the loss of plants attributed to winter injury.

In one year's test the disease was much less severe under a straw covering than without a covering.

Rhizome treatments at transplanting time effectively eliminated the disease if the rhizomes were planted in clean soil.

Various mercury treatments, calomel, Semesan, yellow oxide, mercuric chloride, acid mercury, were all effective.

Some of the formalin treatments were as effective as the acid mercury but further trials are needed before a satisfactory combination of strength of solution and period of immersion can be worked out for all varieties.

Copper sulphate and sulphur in the combinations used were wholly unsatisfactory.

All treatments tested were ineffective in heavily infested soil, although a calomel suspension stabilized with gum arabic gave some promise of effectiveness.

Varieties responded differently to the same treatments.

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LIMA BEAN SEED TREATMENT ON LONG ISLAND[1]

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INTRODUCTION

Lima beans constitute one of the major crops grown on Long Island. The "Fordhook" is used exclusively in commercial plantings and for that reason the work reported in this paper was confined to that variety.

Most of the Limas are grown on soil which is too light for profitable potato production, although a considerable acreage is also grown as a second crop following potatoes. Long Island soils are generally well drained and naturally acid in their reaction. It is rare to find a bean field with a soil reaction higher than pH 5.4 and more often it is well below this figure.

Seed treatment in some form is commonly used with many vegetables as a means of protection against pathogens during the pre-emergence period. With some vegetables seed treatment can be relied upon to improve the stand and less seed per acre can be recommended when treatment is practiced. Until recent years, however, seed treatment has not been recommended for Lima beans.

Work done by Cunningham and Sharvelle (1) indicated that treating with certain organic materials, one of which later was marketed under the trade name of "Spergon," might prove effective in protecting Lima-bean seed under adverse conditions during the pre-emergence period. Later work by McNew (3) has shown that, under western New York conditions, seed treatment of Limas results in improved stands and increased yields. Recently Leach and Holland (2) have reported that seed treatment greatly improves the stand of Lima beans under California conditions.

On Long Island, particularly with early plantings, poor stands of Lima beans are the rule rather than the exception. An effort was made to find some dependable seed protectant which could be recommended for use with this crop.

METHODS

All of the materials used in the experiments were in the form of dusts and were used at the rate of 1.5 ounces per bushel of seed, with the single exception of yellow Cuprocide which was used at half that dosage.

Small plats were used in all tests made at the Research Farm, Riverhead, L. I. The number of seeds per plat and the size of the plat varied in different seasons, depending upon the number of materials used and the amount of land available. In no case was fewer than 50 seeds planted in each plat. With the exception of the 1943 tests all seed was planted by hand with uniform spacing in the row. These small plat tests were used

[1] Journal Paper No. 589, New York State Agricultural Experiment Station, Geneva, N. Y.

as a basis for determining the value of any given material as a seed protectant for Lima beans.

For three years this work was supplemented by machine-planted tests on farms of growers in Suffolk county, on the eastern end of Long Island. These tests were confined to such materials as showed some promise as a seed protectant and were available on the market for the use of growers. In

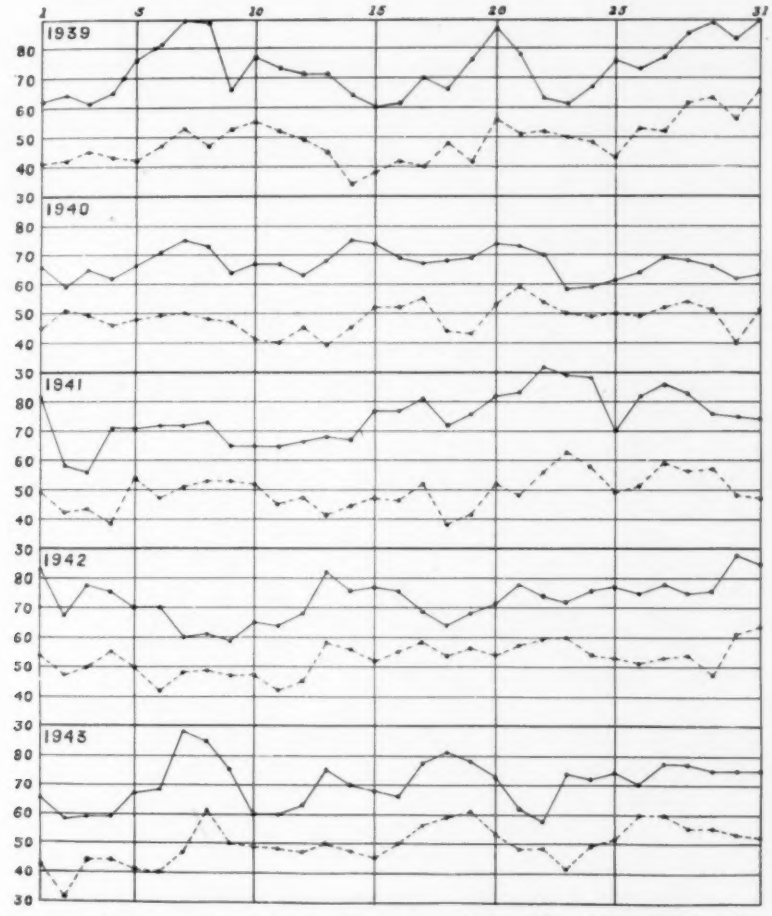

FIG. 1. Maximum and minimum temperature during May, 1939–43 inclusive.

1939 these tests were single-row plats, but in 1940–41 they consisted of half-acre contiguous plats in the same field.

The value of the data was determined by the analysis of variance method. Because of the nonuniformity of size of plats and rate of seeding the stand data are given in per cent rather than actual stand. Yield data are expressed in bushels per acre. In both cases the data represent the average of the number of replications used in the tests. Significant figures are marked with an asterisk.

RESULTS AND DISCUSSION

Any experimental work done under field conditions and covering a period of years will of necessity be carried on under varying climatic conditions. Seasonal conditions vary from year to year and planting dates are subject to local influences. For this reason it is necessary to consider climatic factors in this discussion and the data given indicate that there is a very definite relation between these factors and the results obtained from seed treatment of Lima beans.

The amount of moisture present in the soil at planting time, which is in itself dependent upon rainfall, plays an important role in germination and emergence of Lima beans under Long Island conditions where shallow planting is the general practice. High winds are common and the soil dries rapidly. It is not uncommon to see a marked difference in the time of emergence in a portion of a field planted in the morning as compared with a portion of the same field planted in the afternoon, a difference which can only be accounted for by loss of soil moisture during the day. Low soil moisture at planting time delays germination and emergence. Rainfall immediately following planting, provided it is not too heavy, will offset this condition to some extent but the longer the time elapsing between planting and the first rainfall, the longer will be the pre-emergence period.

The planting season on Long Island is from about May 1 to July 10 and at any time during this period the number of days necessary for emergence is largely dependent upon temperature and rainfall. During the first week in May the temperature (Fig. 1) is normally too low for quick emergence and a period of two weeks or more may elapse between the time of planting and the appearance of seedlings. Later in the season the temperature is usually sufficiently high for rapid germination and emergence. Low soil moisture at planting time, followed by inadequate rainfall, invariably lengthens the pre-emergence period regardless of temperature. The pre-emergence period will also be lengthened if the soil becomes packed because of too heavy rainfall. Observation over a period of years has shown that the ultimate stand of Lima beans is reduced by any delay in emergence and that both temperature and rainfall have a direct bearing on the results obtained from seed treatment.

At the Research Farm, with the exception of the year 1939, no significant difference in stand was obtained from seed treatment in plantings made after June 1. In all of these late plantings the soil moisture was either ample at planting time or rain fell shortly after planting and the temperature was high.

The year 1939 was one of the driest on record. At the time of the first two plantings soil moisture was low and subsequent rainfall was light (Fig. 2). Under these conditions both Spergon and Semesan Jr. significantly increased the stand. At the time of the last planting the soil moisture was at a somewhat higher level but the temperature was high and no

rain fell for eleven days after planting. All of the materials used were effective under these conditions and the increase in stand was highly significant (Table 1).

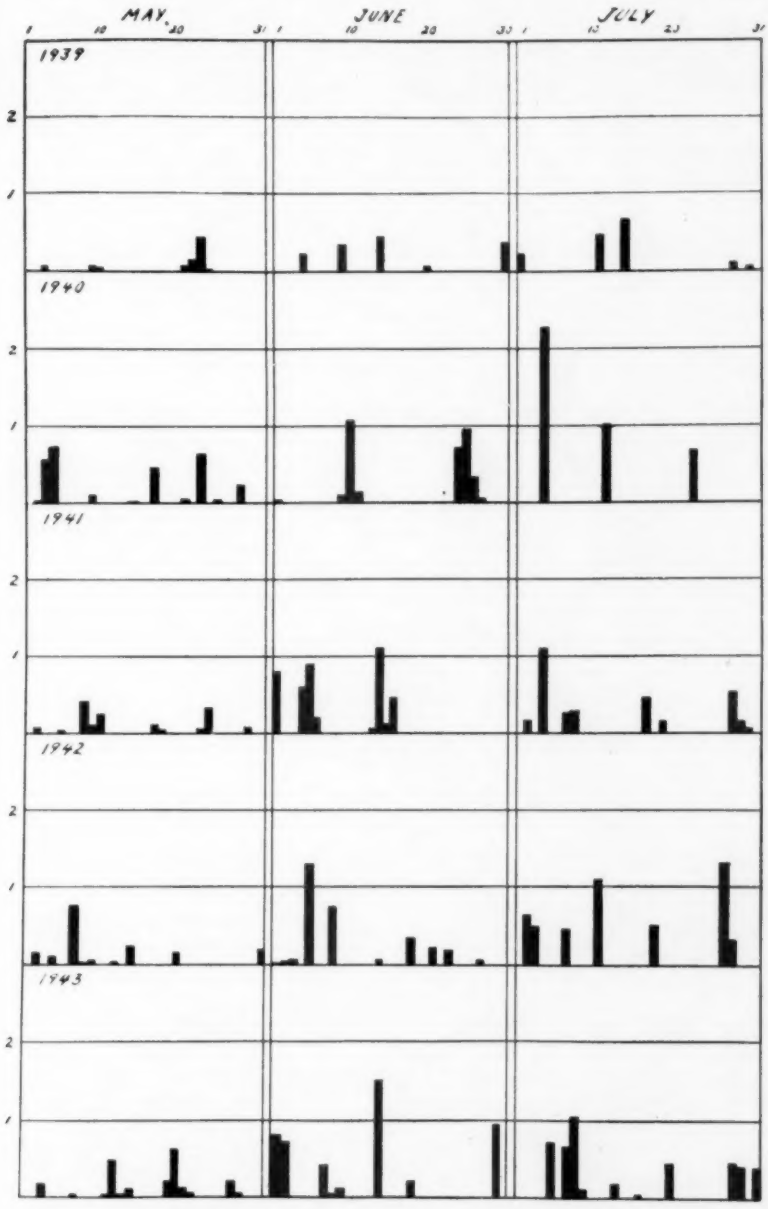

FIG. 2. Rainfall in inches for May, June, and July, 1939–43 inclusive.

On farms of growers in 1939 the results were not so favorable for seed treatment (Table 2). These tests were in widely scattered localities and

TABLE 1.—*The percentage stand and the yield in bushels an acre of Lima beans after seed treatment, at Research Farm, Long Island*

Date planted	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Number of replicates	
1939	Not treated		Spergon		Semesan Jr.		Red Cuprocide		Yellow Cuprocide		Yield	Stand
May 8	67	62	84*	111*	78*	65	74	80*	58	48	4	4
June 3	53	45	72*	68	75*	55	49	43	37	28	4	4
July 1	33	9	85*	41*	86*	17	82*	25*	.....	.....	4	4
1940	Not treated		Spergon		Semesan Jr.							
May 13	51	.....	60	.....	49	.....					4	...
29	63	.....	83*	.....	79*	.....					4	...
June 7	52	32	67	31	69	36					4	4
12	81	43	89	76	81	51					4	4
20	89	130	91	163	95	148					4	4
July 3	87	157	87	147	87	156					4	4
1941	Not treated		Spergon		Semesan Jr.		Copper oxychloride sulfate					
May 6	74	209	91*	204	92*	188	67	136			4	4
16	90	146	95	177	97*	129	72*	141			4	4
27	91	228	91	186*	91	213	78*	200			4	4
June 6	93	241	89	215	88	230	87	244			4	4
1942	Not treated		Spergon		Semesan Jr.		Fermate					
May 11	44	163	48	162	43	124	47	177			6	6
21	73	143	78	131	77	125*	77	127*			6	6
June 13	87	.....	88	.....	85	.....	90	.....			3	...
29	69	.....	77	.....	67	.....	73	.....			3	...
July 6	66	.....	72	.....	67	.....	73	.....			3	...
7	68	.....	65	.....	72	.....	70	.....			3	...
1943	Not treated		Spergon		Semesan Jr.		Arasan					
May 18	62	.....	75*	.....	64	.....	.....	.....			6	6
25	79	181	82	185	79	166	.....	.....			6	6
June 4	86	175	89	185	88	201	.....	.....			6	6
11	84	105	88	129	87	96	87	113			6	6

NOTE: Figures marked * are significant as compared with untreated check.

undoubtedly the results were influenced by local variations in temperature and rainfall. Spergon improved the stand in three of eight plantings. Red Cuprocide was beneficial in two cases and detrimental in two others. Yellow Cuprocide reduced the stand in six plantings and the figures are significant.

In 1940 the first planting at the Research Farm was in soil with a high moisture content and rain fell shortly after planting. Both Spergon and Semesan Jr. failed to give any significant difference in stand. At the time of the second planting on May 29 soil moisture was low and no rain fell for nearly two weeks after planting. Under these conditions all of the materials used significantly increased the stand.

Tests made on farms of growers in 1940 were not too favorable for seed treatment. Spergon increased the stand in nine plantings and actually decreased it significantly in three of twenty-one tests. In the same number of tests Semesan Jr. increased the stand in four cases and decreased it in three. In most cases where the stand was increased by seed treatment it occurred in the earliest plantings made in a locality.

In 1941 the rainfall during April was very light and on May 6, when the first planting was made at the Research Farm, the soil was very dry. Rain fell two days later with the result that the stand from untreated seed was unusually good for so early a planting. The stand was significantly improved, however, where the seed was treated with Spergon and Semesan Jr. At the time the second planting was made soil-moisture conditions were more favorable for germination and Semesan Jr. was the only material used which improved stand.

Tests with growers in 1941 were confined to the use of Spergon. All of these plantings were made prior to June 1 and seed treatment increased stands significantly in four of the earliest plantings.

No improvement resulted from seed treatment in 1942. Low temperature and high soil-moisture content resulted in a poor stand in the planting made on May 11. Seed treatment did not improve the stand and it was apparent that under such conditions none of the materials used gave sufficient protection to be of any value.

Seed treatment with Spergon was of value in increasing the stand in the first planting made in 1943. There was ample soil moisture at planting time and the temperature was sufficiently high for good germination.

Yield records were not taken on all seed treatment tests but where they were taken (Tables 1 and 2) the figures show that in very few cases was the yield influenced by differences in stand due to seed treatment. At the Research Farm during the extremely dry season of 1939 the differences in stand were sufficiently large to be reflected in increased yields.

Special mention should here be made of the copper compounds used. Red Cuprocide has some value as a seed protectant for Lima beans under certain conditions but unfortunately causes a hardening of the seed coat and stunting of the seedlings which more than offsets its value as a seed

TABLE 2.—*The percentage stand and the yield in bushels an acre of Lima beans after seed treatment, on farms of growers on Long Island*

Date planted	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Stand %	Yield Bu.	Number of replicates	
1939	Not treated		Spergon		Red Cuprocide		Yellow Cuprocide		Stand	Yield
May 9	43	173	47*	178	44	148*	37*	139*	8	4
9	39	180	47*	170	47*	129*	44	180	8	4
27	45	149	49	149	47	182*	36*	134	8	4
June 3	68	162	67	153	57*	163	55*	147	8	4
5	38	129	45*	154	48*	150	23*	68*	8	4
12	95	237	95	234	94	255	90*	231	3	3
19	49	106	51	86*	45*	103	44*	77*	8	4
20	47	23	51	49	50	28	49	.....	8	4
1940	Not treated		Spergon		Semesan Jr.					
May 10	69	139	78*	173	67	180			8	4
10	91	185	93	142*	93	174			5	4
10	51	.....	60*	.....	66*	.....			10	...
14	63	123	67	154	64	138			10	4
15	65	.....	69	.....	71	.....			8	4
18	27	.....	30	.....	36*	.....			8	...
18	92	137	80*	178	92	140			8	4
20	80	113	68*	154	69*	170			8	4
30	85	.....	75	.....	85	.....			4	...
30	84	.....	80*	.....	79*	.....			8	...
30	67	.....	81*	.....	60	.....			4	...
June 4	77	.....	82*	.....	77	.....			8	...
4	67	.....	73*	.....	73*	.....			10	...
7	58	.....	63*	.....	57	.....			4	...
12	94	.....	93	.....	96	.....			5	...
18	62	.....	69*	.....	68	.....			4	...
20	56	37	67*	39	61	36			10	4
20	51	161	54	202*	55	178*			8	4
20	48	.....	45	.....	43*	.....			8	...
28	73	.....	.....	.....	79*	.....			8	...
29	75	.....	79*	.....	.....	.....			8	...
July 2	78	.....	.....	.....	78	.....			6	...
3	76	.....	76	.....	.....	.....			6	...

TABLE 2.—(*Continued*)

Date planted	Stand %	Yield *Bu.*	Stand %	Yield *Bu.*		Number of replicates	
1941	Not treated		Spergon			Stand	Yield
April 30	33	.....	63*	.....		10	..
May 12	78	191	84*	182		20	10
12	81	121	90*	107		12	8
13	93	134	98	153		10	6
16	87	267	96*	280		20	10
19	95	204	86	218		8	8
19	83	193	79	185		20	10
27	94	201	96	171*		12	10

NOTE: Figures marked * are significant as compared with untreated check.

protectant. This hardening and stunting effect is even more marked with both Yellow Cuprocide and copper oxychloride sulfate.

SUMMARY AND CONCLUSIONS

Seed-treatment experiments with Lima beans were carried on at the Long Island Vegetable Research Farm for five years. For three years during this period extensive tests were conducted on farms of growers. Under conditions of low soil moisture and low rainfall following planting the stand was improved regardless of temperature. Under conditions of high soil moisture and low temperature none of the materials used gave sufficient protection to be of commercial value. Except in an unusually dry season the increase in stand obtained by seed treatment was not sufficient to influence the yield.

Treatment with copper compounds hardened the seed coat and stunted the plants.

From the data presented it is concluded that on Long Island seed treatment of Lima beans has some value as a seed protectant and may be expected to improve the stand under conditions of low soil moisture at planting time followed by a period of low rainfall. The stand in plantings made in early May, when the temperature is normally low, will usually be increased as a result of seed treatment, provided the rainfall is not too heavy during the pre-emergence period.

In general, most of the materials used in these tests improved stand under conditions favorable to results from seed treatment although in some cases the number of tests was limited and the results in such cases can only be considered as indicative. Of the materials used, Spergon showed the most promise as a seed protectant for Lima beans.

On the basis of these experiments seed treatment of Lima beans can not be recommended to Long Island growers as a profitable practice.

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EFFECT OF SEVERAL SEED PROTECTANTS ON GERMINATION AND STANDS OF VARIOUS FORAGE LEGUMES[1]

J. Lewis Allison and J. H. Torrie[2]

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During the last few years recommendations have been made for the general use of seed protectants. However, little experimental evidence has been presented to date with respect to the possible effect of protectants on seed germination and subsequent stands of small seeded forage legumes. Chilton and Garber[3] summarized the literature through 1940 and presented data on the effect of seed treatment on stands of some forage legumes at State College, Pennsylvania. In greenhouse tests they obtained significant increases in stand with certain fungicides, using certain species of legumes. Other species did not respond to the various fungicides tried, and seeds of some species were injured. Kreitlow[4] reported increases in stands of alfalfa and red clover in field tests in Pennsylvania when the seed had been treated with certain fungicides. This paper presents the results from experiments at Madison, Wisconsin, in the greenhouse and the field during 1942–43.

MATERIALS AND METHODS

Medicago sativa (alfalfa), *Melilotus alba* (white-flowered sweet clover), *Trifolium pratense* (medium red clover), *Trifolium hybridum* (alsike clover), *Trifolium fragiferum* (strawberry clover) and *Trifolium repens* (ladino clover) were selected from the small-seeded legumes of commercial importance. The protectants tested were 5 per cent ethyl mercury phosphate (New Improved Ceresan), tetrachloro-parabenzoquinone (Spergon), and 50 per cent tetra-methyl thiuramdisulfide (Arasan). These dusts represent an organic mercuric and two organic, nonmercuric compounds.

A standard germination test was made on the seeds of all species tested. A 10-gram sample of each species was weighed, treated by adding an excess of protectant, shaken well, screened and re-weighed. The weight dosage was the difference in weight of the untreated and treated lots expressed in percentage of untreated seed. One hundred seeds of each treated lot and one untreated check lot of each species were plated on nutrient agar immediately following treating and again after storage for 30 days in corked glass vials.

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Forage Crops and Diseases; Bureau of Plant Industry, Agricultural Research Administration, U. S. Dept. of Agriculture. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] Associate Pathologist, Division of Forage Crops and Diseases, and Assistant Professor of Plant Pathology; and Assistant Professor of Agronomy, respectively.

[3] Chilton, S. J. P., and R. J. Garber. Effect of seed treatment on stands of some forage legumes. Jour. Amer. Soc. Agron. 33: 75–83. 1941.

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In the greenhouse two different types of soil were used. Compost of pH 7.1 known to be infested with *Pythium, Rhizoctonia* and *Fusarium* spp., which cause damping-off of legume seedlings, and woods-humus of pH 6.4 which was free of damping-off organisms. The design used was a split-plot with 3 replications. The replicates were divided into blocks of 4 rows each. A block consisted of the 3 treatments and an untreated check of each species. The treatments were randomized within replicates. The rows were 20 inches long and 2 inches apart. The seed was planted immediately following treating at a uniform depth of one-half inch at the rate of 50 seeds per row. The temperature ranged between 60° and 70° F. Water was applied as a fine spray often enough to keep the surface of the soil moist.

A field experiment of the same design but on a larger scale was planted in early May, 1943, on land on which forage legumes had been in the rotation for many years. The rows, of 200 seeds, were 4 feet long and 6 inches apart. No attempt was made to alter environmental conditions.

Plant emergence counts were made on alternate days until maximum emergence for each species was determined. Final stand counts were made after all post-emergence damping-off had ceased, usually six days after total emergence. Post-emergence damping-off is defined as the death of fully emerged seedlings prior to the formation of the first unifoliate leaf and pre-emergence damping-off as the death of germinating seedlings prior to emergence. All seedlings were removed from the soil and examined for nodule development 5 weeks after planting.

EXPERIMENTAL RESULTS

Ten-gram seed lots of each species retained an equivalent amount, approximately one per cent by weight, of each of the three fungicides.

No injurious effect on germination was apparent when the treated seed of any species was compared with untreated seed in the agar plate test. However, the germination of ladino and strawberry clovers was retarded but not reduced in total when treated with Spergon. The results were the same when this test was repeated after storing seed samples for 30 days.

Table 1 summarizes the effects of the seed protectants on the total emergence and final stand of the legumes when grown in compost in the greenhouse. The differences in the values for total emergence and final stand give a measure of post-emergence damping-off. Table 2 summarizes the results obtained when the legumes were grown in woods-humus in the greenhouse. Table 3 summarizes the results of the field experiment. As there was no post-emergence damping-off with any species in woods-humus or in the field, total emergence and final stand values are the same.

The effects of the protectants were pronounced for all species in compost. All of the protectants resulted in significant increases in the total emergence of alfalfa and alsike clover. Only New Improved Ceresan significantly increased the total emergence of sweet clover. The total emergence of red clover was significantly increased by New Improved Ceresan and Arasan.

TABLE 1.—*Effects of three seed protectants on total emergence and final stand of six forage legumes grown in compost, infested with damping-off organisms, in the greenhouse, at Madison, Wisconsin*

Legumes	Germination	Total emergence,[a] number of plants[b]				Final stand,[a] number of plants[c]			
		Check	New Improved Ceresan	Arasan	Spergon	Check	New Improved Ceresan	Arasan	Spergon
	%								
Medicago sativa	75	309	424**	369**	387**	191	310**	264**	251**
Melilotus alba	60	241	287*	279	263	188	228	211	218
Trifolium pratense	60	358	434**	402*	358	315	394**	362*	324
Trifolium hybridum	60	180	278**	253**	232*	149	241**	220**	195*
Trifolium fragiferum	65	338	329	362	205**[d]	324	317	334	184**
Trifolium repens (ladino)	55	194	170	180	67**[d]	160	155	169	60**

[a] Four experiments, 12 replications.

[b] M.S.D. 1% = 56; 5% = 42. Figures significant at the 1% level are marked with a double asterisk; those significant at the 5% level, with a single asterisk.

[c] M.S.D. 1% = 57; 5% = 43. Figures significant at the 1% level are marked with a double asterisk; those significant at the 5% level, with a single asterisk.

[d] Emergence retarded.

TABLE 2.—*Effects of three seed protectants on total emergence and final stand of six forage legumes grown in woods-humus in the greenhouse, at Madison, Wisconsin*

Legumes	Germination	Total emergence and final stand,[a] number of plants[b]			
		Check	New Improved Ceresan	Arasan	Spergon
	%				
Medicago sativa	75	109	112	119	108
Melilotus alba	60	92	113**	120**	104
Trifolium pratense	60	92	92	86	96
Trifolium hybridum	60	91	78	86	77
Trifolium fragiferum	65	99	86	87	87[c]
Trifolium repens (ladino)	55	82	90	100*	97*[c]

[a] One experiment, 3 replications.
[b] M.S.D. 1% = 19; 5% = 14. Figures significant at the 1% level are marked with a double asterisk; those significant at the 5% level, with a single asterisk.
[c] Emergence retarded.

Spergon delayed emergence and significantly decreased the total emergence of strawberry and ladino clovers. None of the protectants had any effect in controlling post-emergence damping-off of any species. The effect of the fungicides on total emergence and final stand remained approximately the same for all species.

The effects of the fungicides were not pronounced for all species in woods-humus. None of the protectants had any effect on alfalfa, red clover and alsike clover. New Improved Ceresan and Arasan significantly increased the total emergence and final stand of sweet clover. Spergon retarded emergence but did not significantly decrease the total emergence or final stand of strawberry clover. Arasan and Spergon significantly increased the total emergence and final stand of ladino clover, although use of Spergon resulted in retarded emergence.

TABLE 3.—*Effects of three seed protectants on total emergence and final stand of six forage legumes in a field experiment at Madison, Wisconsin*

Legumes	Germination	Total emergence and final stand[a], number of plants			
		Check	New Improved Ceresan	Arasan	Spergon
	%				
Medicago sativa	75	354	309	325	353
Melilotus alba	60	251	219	194	224
Trifolium pratense	60	195	252	254	242
Trifolium hybridum	60	230	231	217	241
Trifolium fragiferum	65	293	256	238	252[b]
Trifolium repens (ladino)	55	318	286	259	285[b]

M.S.D. 1% = 85; 5% = 64.
[a] One experiment, 3 replications.
[b] Emergence retarded.

In the field none of the fungicides had any statistically significant effect on the total emergence or final stand of any species. Spergon, however, retarded the emergence of strawberry and ladino clovers.

Five weeks after the date of planting, the seedlings of all species in each experiment were removed from the soil and examined for nodule formation. When compared with the untreated checks, nodule development was normal for all species regardless of treatment.

DISCUSSION AND SUMMARY

The effects of three seed protectants on germination and stands of six forage legumes were studied in laboratory, greenhouse, and field experiments. Approximately one per cent by weight of each fungicide adhered to the seed of each legume. This is a heavy dosage when compared with the recommended rates of application for cereal and vegetable crop seeds. However, laboratory tests demonstrated that none of the protectants had an apparent injurious effect on germination. One fungicide, Spergon, retarded but did not reduce the germination of strawberry and ladino clovers but had no such effect on alsike clover, medium red clover, alfalfa or white-flowered sweet clover. Retardation was further accentuated when strawberry and ladino clovers were planted in different types of soil. In humus soil in the greenhouse and in the field where total emergence and final stand remained the same because no post-emergence damping-off occurred, strawberry and ladino clovers were retarded in emergence but not reduced in total emergence. However, when planted in compost known to be infested with damping-off organisms, strawberry and ladino clovers were retarded in emergence and very significantly reduced in total emergence, indicating that the protectant so inhibited germination that necessary damping-off organisms had added opportunity to cause pre-emergence damping-off or rotting of the seedlings.

In compost infested with damping-off organisms certain species were protected against pre-emergence damping-off by certain of the fungicides but none of them had any effect in controlling post-emergence damping-off with any species.

In humus soil only white-flowered sweet clover and ladino clover were benefited by any of the fungicides, and as there was no damping-off the results are interpreted as being due to a stimulatory effect of the protectants on seed germination of the two species.

In the field none of the fungicides was found to be significantly beneficial with any legume treated.

From the results obtained in all experiments it was apparent that none of the fungicides used inhibited nodule formation. Studies are in progress on the effects of adding nodule bacteria to seed treated with fungicides.

The relative value of seed treatment as a means of increasing germination and bettering stands of the small seeded forage legumes can be determined only by making many experiments in numerous localities. The ulti-

mate proof of the value of fungicides for these legumes would be the general adoption of seed treatment based upon sound experimental evidence. There would seem to be little evidence from these experiments to recommend such general adoption in Wisconsin.

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CROP ROTATION AND SOIL DRAINAGE EFFECTS ON SUGAR BEET TIP ROT AND SUSCEPTIBILITY OF OTHER CROPS TO APHANOMYCES COCHLIOIDES[1]

W. F. BUCHHOLTZ[2]

(Accepted for publication April 12, 1944)

The pathogenicity of *Aphanomyces cochlioides* Drechsler to tap roots of sugar beets has been reported.[3] The abundance of tip rot in some sugar beet fields and its scarcity or near absence in others in Hancock County, Iowa, in 1937, 1938, and 1939 led to an examination of the crop sequence records of such fields and to a determination of susceptibility to *A. cochlioides* of other crops grown in rotations with beets. The presence or absence of severe tip rot was also found to be associated with the locations of tile drains.

CROPS RESISTANT TO APHANOMYCES COCHLIOIDES

Crops commonly grown in rotation with sugar beets in northern Iowa are corn, oats, soybeans, alfalfa, and sweet clover. Seedlings of these crops, together with sugar beets, were exposed to *A. cochlioides* in the following manner. Soil in 36 glazed one-gallon crocks was steamed at 20 pounds' pressure for one hour. Ten-day-old cultures of two isolates of *A. cochlioides* growing on corn-meal agar were cut into centimeter squares and uniformly distributed on a plane about two inches below the soil surface in 24 crocks, at the rate of one Petri dish culture per crock. Plain corn-meal agar was likewise placed in 12 crocks. Seeds of corn (7), oats (21), soybeans (14), and sugar beets (14 clusters) were distributed uniformly on a plane one-half inch deep. There were six crock cultures for each crop, two noninfested (corn-meal agar only) checks and four cultures infested with *A. cochlioides*, two by each of two isolates. Seedling stands were recorded 12 and 17 days after planting. Final stand, length and weight of tops and roots, and presence of lesions on the roots were recorded 27 days after planting.

In the four infested crock cultures planted with sugar beets, only two seedlings remained after 27 days, and these were diseased. *Aphanomyces cochlioides* was isolated from four seedlings that damped off. In the two noninfested crock cultures, 32 and 23 healthy beet seedlings remained. The stands of the other five crops were uniformly good, and no variation in length or weight of tops and roots occurred. Numerous lesions were present on the roots of corn and soybeans grown in infested and in noninfested soils, but attempts to isolate *A. cochlioides* from these lesions failed. A repetition of this experiment yielded essentially the same results.

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[2] Plant Pathologist, South Dakota Agricultural Experiment Station, formerly research assistant, Botany and Plant Pathology Section, Iowa Agricultural Experiment Station. The author is indebted to Dr. I. E. Melhus for aid and suggestions during the course of investigations and preparation of the manuscript.

[3] Buchholtz, W. F., and C. H. Meredith. Pathogenicity of *Aphanomyces cochlioides* on tap roots of the sugar beet. Phytopath. 34: 485–489. 1944.

TABLE 1.—*Crop sequences of fields in northern Iowa with different amounts of sugar beet tip rot in 1937, 1938, and 1939*

Fields 1, 2 (Nor. Iowa Exp. Assoc., Kanawha)

	1		2
1930	Beets	1930	Beets
1931	Small grain	1931	Beets
1932	Beets	1932	Corn
1933	Corn	1933	Oats seeded to alfalfa
1934	Barley	1934	Alfalfa
1935	Beets (poor crop)	1935	Alfalfa
1936	Corn	1936	Beets (normal crop)
1937	Beets	1937	Corn
		1938	Beets
Tip rot	Crop failure because of tip rot	Tip rot	Crop failure because of tip rot

Field 3 (N. Christians, Kanawha)

	A	B
	No beets prior to 1934	
1934	Oats	Beets
1935	Alfalfa	Corn
1936	Alfalfa	Beets
1937	Alfalfa	Corn
1938	Beets	Beets
Tip rot	None	90 pct.

Field 4 (M. Hill, Clarion)

	A	B	C
1932	Beets	Beets	Beets
1933	Corn	Corn	Corn
1934	Oats	Oats	Oats
1935	Sweet clover	Sweet clover	Alfalfa
1936	Corn	Beets	Alfalfa
1937	Corn	Corn	Alfalfa
1938	Oats	Oats	Alfalfa
1939	Beets	Beets	Beets
Tip rot	None	50 pct.	None

Field 5 (F. L. Bubeck, Clarion)

	A	B
1932	Alfalfa	No beets
1933	Corn	No beets
1934	Corn	Corn
1935	Oats	Beets
1936	Beets	Corn
1937	Corn	Corn
1938	Beets	Beets
Tip rot	60 pct.	20 pct.

TABLE 1—(*Continued*)

Fields 6, 7 (A. Christians, Kanawha)		
	6	7
1932	Pasture	No beets
1933	Corn	No beets
1934	Oats	Beets
1935	Beets	Corn
1936	Corn	Oats
1937	Oats	Sweet clover, alsike, timothy
1938	Beets	Beets
Tip rot	70 pct.	10 pct.

Field 11 (D. Waggoner, Kanawha)	
1933	Beets
1934	Oats
1935	Corn
1936	Oats
1937	Sweet clover
1938	Beets
Tip rot	95 pct.

Field 13 (Nor. Iowa Exp. Assoc., Kanawha)	
1930	Beets (seedlings killed)
1931	Corn
1932	Beets (failure: tip rot)
1933	Corn
1934	Flax
1935	Alfalfa
1936	Alfalfa
1937	Alfalfa
1938	Alfalfa
1939	Beets
Tip rot	None

Fields 8, 9, 10 (N. E. Schroeder, Clarion)			
	8	9	10
1933	Beets	No beets prior to 1937	
1934	Oats		
1935	Sweet clover		
1936	Beets		
1937	Corn	Sweet clover	Sweet clover, alfalfa
1938	Beets	Beets	Beets
Tip rot	50 pct.	None	None

Field 12 (H. Christians, Kanawha)	
	No beets prior to 1935
1935	Beets
1936	Corn
1937	Oats
1938	Sweet clover
1939	Beets
Tip rot	None

Barley, red clover, flax, sugar beets, pigweed (*Amaranthus retroflexus* L.), and lamb's quarters (*Chenopodium album* L.), the latter two close relatives of the sugar beet, were exposed to *A. cochlioides* in two experiments identical to the one described except that pots were used instead of crocks. Barley and red clover were not parasitized by *A. cochlioides*. In the first experiment flax germination apparently was interrupted by *A. cochlioides*, as the stand was less in infested soil than in noninfested soil; but this difference did not occur in the second trial. All seedlings of sugar beet, pigweed, and lamb's quarters grown in infested soil were infected, and *A. cochlioides* was isolated from one or more seedlings of each. All seedlings grown in noninfested soil were healthy. The susceptibility of pigweed and lamb's quarters to *A. cochlioides* and their general distribution in northern Iowa may account for the presence of this organism in northern Iowa soils. In many experiments with steamed soil, there has been no evidence that *A. cochlioides* is seed-borne.

OCCURRENCE OF TIP ROT IN FIELDS WITH KNOWN CROP SEQUENCES

The crop sequences of two adjoining fields (1 and 2) on the Northern Iowa Experimental Association Farm at Kanawha that failed because of tip rot in 1937 and 1938 and the sequences of various commercial fields showing severe, slight, or no damage from tip rot in 1938 or 1939 are recorded in table 1.

In the commercial fields large samples could not be removed for loss estimates. Usually two people visited the fields and agreed upon an estimate of the damage after examining a few beets from each field under observation. Although these estimates may not be accurate it can be said that clear-cut differences in the amount of tip rot existed and that they were in the direction of and in proportion to the estimates recorded.

In fields 3, 4, and 5, the various portions with different crop sequences were distinguished by different amounts of tip rot (Fig. 1). Such differences were apparent across the entire field and virtually to the row.

The most striking case observed was field 3. Part B was a virtual failure because of tip rot. It was in beets for the third time in five years. The remainder of the field (A) was in beets for the first time and had been preceded by alfalfa; it was an exceptionally good crop and free of tip rot. The two portions of this field are shown in figure 1. Field 4 was of special interest because it was in three parts. Part B was in beets the third time in eight years, with only two crops, corn and oats, intervening between the 1936 and 1939 beet crops. This part of the field suffered a 50 per cent loss from tip rot. Parts A and C, without the 1936 beet crop, had no noticeable amount of tip rot. In field 5, part A had only one corn crop between beet crops and an estimated 60 per cent of tip rot; part B had two intervening corn crops, with an estimated 20 per cent of tip rot.

The principal difference between fields 6 and 7 was the extra crop year (sweet clover, alsike, timothy) between sugar beet crops in field 7, with

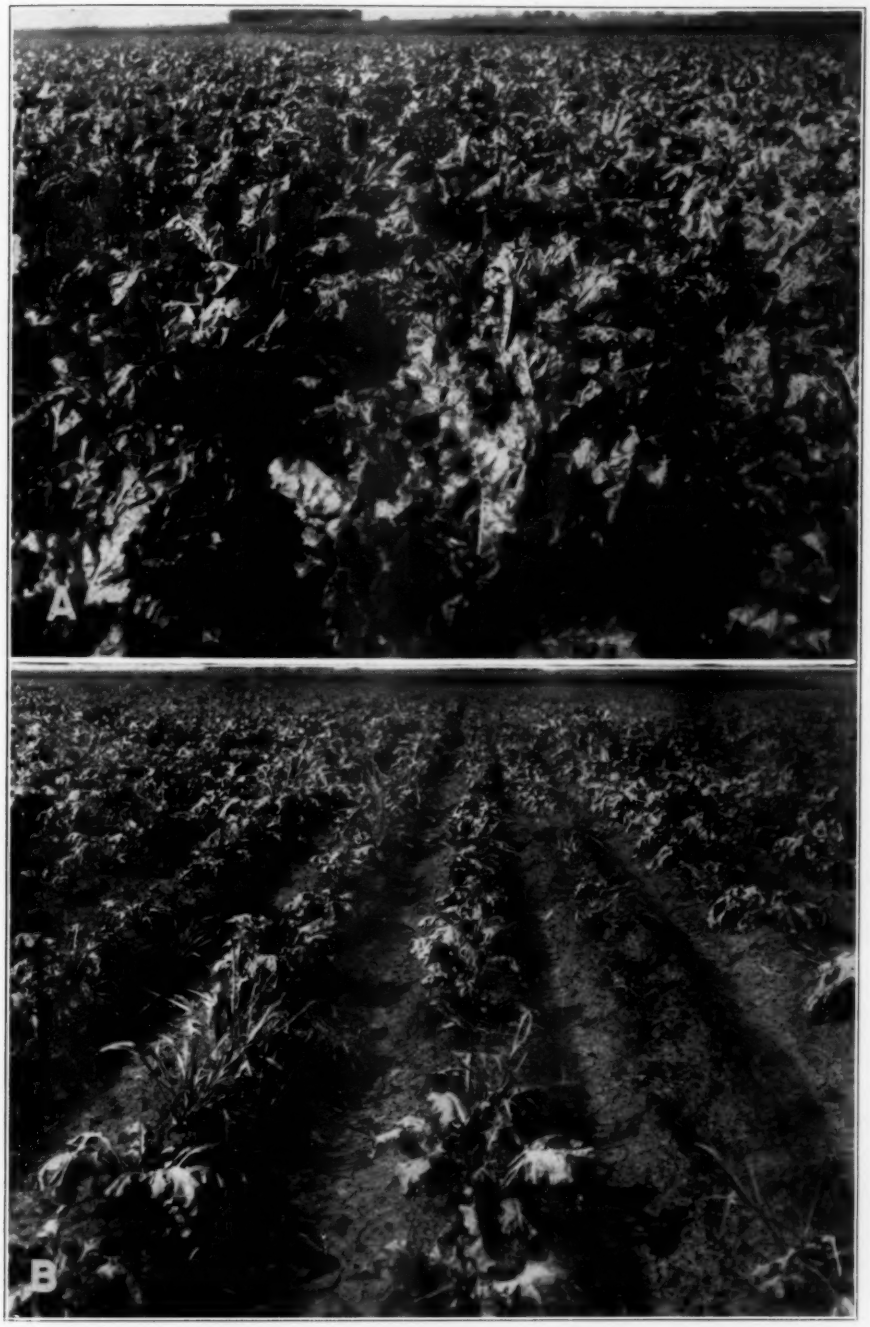

FIG. 1. A. Tip-rot-free portion of field 3, which was in beets for the first time and had previously been in alfalfa. B. Beet failure because of tip rot in another portion of field 3. This was the third crop of beets in five years, with two intervening crops of corn.

also much less tip rot. Field 8 had three crops of sugar beets in six years, with only one intervening corn crop between the last two beet crops. The second (1936) beet crop, according to the owner, was a normal one. The third (1938) beet crop suffered an estimated 50 per cent loss from tip rot. Fields 9 and 10 on the same farm were producing their first beet crop and had no tip rot. Field 11 is recorded because it seems to be a glaring anomaly. There were four intervening crops between the 1933 and 1938 beet crops, yet almost a complete loss from tip rot occurred. This field, however, was the only bad case of tip rot observed on other than Clarion or Webster soils; it was of the Fargo series. Field 12, in sharp contrast, had no tip rot, with only three crops (corn, oats, sweet clover) intervening between beet crops.

In five other fields, two in 1938 and three in 1939, sugar beets were growing on a piece of land for the first time. All five fields were free of tip rot.

In three cases (Fields 3B, 4B, 8), sugar beets occurred on the land three times in eight years or less. In all three fields, the third crop was severely tip rotted. In three cases (Fields 3A, 4C, 13), sugar beets followed three or four years of alfalfa; in none of the three was tip rot found. In one of these (Field 13), at least one previous beet crop had been a failure because of tip rot.

These general statements are ventured regarding the appearance of tip rot under various crop sequences: (1) Tip rot did not occur in the first crop of beets on a given piece of land. (2) The third crop of beets in 8 years or less (Fields 3B, 4B, 8) was severely tip rotted. (3) There was no indication of severe tip rot after alfalfa (Fields 3A, 4C, 13), even in one field where tip rot had at one time previously occurred (Field 13).

It seems therefore that a four-year rotation should prove adequate for the most part to prevent severe losses from tip rot. In a field where tip rot is serious, however, an interval of six or more years may be necessary to "clean up" the land for the next beet crop, including perhaps a three- or four-year period devoted to alfalfa, as in field 13.

TIP ROT IN RELATION TO TILE DRAINS

An unusual distribution of tip rot in field 2, 1938, was determined to be coincident with the distribution of tile drains. Three areas, 45×375, 33×375 and 27×375 ft, had, by actual count, a rather uniform 75 per cent stand of healthy beets on August 19. In 10-ft. borders around these areas the stand of healthy beets fell off sharply and by actual count was 27.3 per cent. In the rest of the field, the stand of healthy beets was 10.3 per cent. Nearly all the loss of stand was the result of tip rot.

When the presence of tile drains in the center of the relatively tip-rot-free areas was postulated, six holes were dug to determine the presence or absence of tiles. In four locations at the exact centers of the three relatively tip-rot-free areas, tiles were found at a depth of about 5 ft. Tiles were absent in two other locations, one beyond the widest tip-rot-free area and

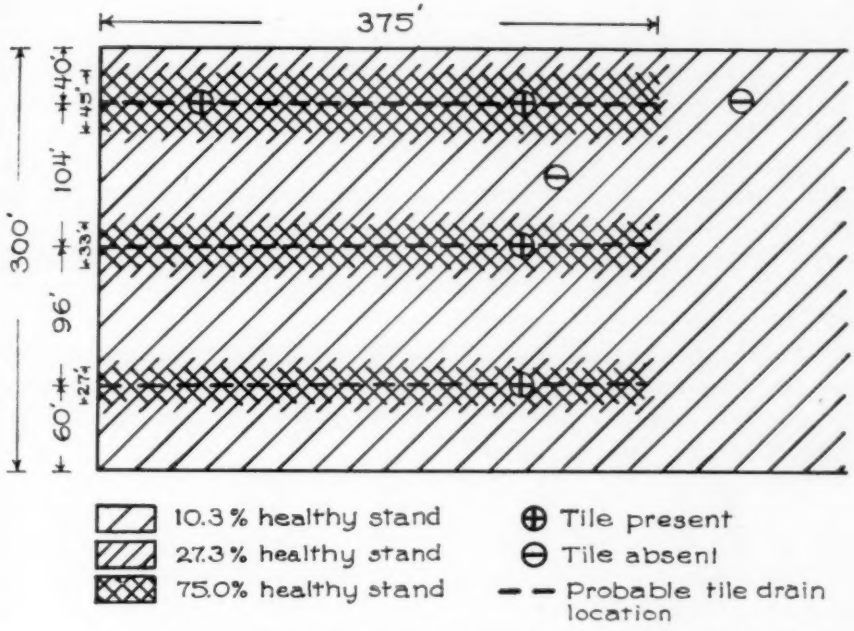

FIG. 2. Locations of tile drains in field with various amounts of tip rot.

exactly in line with the tile drain already located, the other exactly between this tile and the tile located in the adjacent tip-rot-free area.

The positions of the relatively tip-rot-free areas, the postulated locations of tile drains, and the points where tiles were or were not found are shown in figure 2.

Moisture determinations in duplicate were made of soil samples taken from three depths directly over the two south tile drains, between these two, and north of the central line. The results are tabulated in table 2.

The lower moisture content over, as compared with the area between, the tiles is evident. The difference probably is not significant in itself, but these determinations were made when the soil, though not dry, was definitely not water-logged. The farm foreman, who attended to planting and cultivation

TABLE 2.—*Moisture content of soil samples from three depths over and between tile drains, field 2, Kanawha, August, 1938*

Depth (inches)	Percentage moisture					
	Over tile drains			Between tile drains		
	Middle tile	South tile	Average	North of middle tile	South of middle tile	Average
0–6	30.1	28.9	29.5	31.8	33.4	32.6
6–12	28.1	26.5	27.3	28.4	32.5	30.4
12–18	25.5	25.2	25.3	26.0	27.4	26.7

of this field, observed that on several occasions in the spring the areas over the tile drains were dry enough to cultivate before the areas between tiles.

In two other fields near Kanawha less than the field average of tip rot occurred in the areas that the farm operators indicated were over tile drains. The presence or absence of tiles was not determined by digging.

In two greenhouse experiments, sugar beets were grown in steamed soil and in steamed soil infested with *A. cochlioides*, both subjected to limited irrigation from above in contrast to abundant irrigation from above and below. In neither experiment was there a difference in amount or degree of infection of the beets by *A. cochlioides* that could be attributed to the difference in irrigation.

The association of tile drains with less tip rot in field 2 and perhaps two other fields was so clear-cut as to allay any doubts of its existence, but the relationship between tip rot severity and excessive soil moisture, although indicated, was not definitely established.

SUMMARY

The roots of corn, oats, soybeans, alfalfa, sweet clover, barley, and red clover were not infected when grown in soil infested with *Aphanomyces cochlioides*. Germination of flax apparently was interrupted by *A. cochlioides* in one trial, but in the second experiment flax did not appear to be susceptible. The roots of sugar beets, pigweed (*Amaranthus retroflexus* L.), and lamb's quarters (*Chenopodium album* L.) were infected. The susceptibility of these two common weeds suggests the universal presence of *A. cochlioides* in northern Iowa soils.

In fields with varying amounts of sugar beet tip rot the following observations were made: (1) In no case did tip rot occur in the first crop of beets on a given field. (2) The third crop of beets in 8 years or less was severely tip-rotted. (3) There was no indication of severe sugar beet tip rot after alfalfa, even in one field where tip rot had at one time previously occurred.

Intervals of three or four years between beet crops should adequately avoid severe losses from tip rot. However, an interval of six or more years, including three or four years of alfalfa, may be desirable for a field in which sugar beet tip rot has been severe.

Tile drains were definitely associated with less than the field average of sugar beet tip rot at Kanawha in 1938. The relationship between tip rot severity and excessive soil moisture was indicated but not definitely established.

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INFECTION PHENOMENA IN TOMATO-FRUIT ROT CAUSED BY PHYTOPHTHORA CAPSICI[1]

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(Accepted for publication April 25, 1944)

Recent investigations on the tomato-fruit-rot disease in Colorado caused by *Phytophthora capsici* Leonian have disclosed the advisability of using histological studies in current pathological research.[2] Initial invasion, the development of the causal agent in host tissue, and subsequent sporulation of the organism have been studied histologically. Another phase to merit attention was the investigation of an observed difference between stylar- and stem-end tissues to infection.

MATERIALS AND METHODS

Green tomato fruits of the Landreth variety were inoculated by placing drops of water containing active swarmspores on the uninjured surfaces of the fruits. The swarmspore inoculum was prepared by the soil-inoculum technique.[3] The inoculated fruits then were incubated in moist chambers at 25° C. for varying times.

After incubation periods of 2 to 72 hours, small pieces for histological studies were cut from the areas where the swarmspores had been placed. These pieces were killed and fixed in formalin-acetic-alcohol and in several of the chrom-acetic and chrom-acetic-formalin solutions described by Sass.[4] The "Craf" I and "Craf" II formulae gave the best results and consequently were used most frequently. Tertiary butyl alcohol was the best dehydrating agent when used according to Johansen's method[5] as modified by Sass.[4] Ethyl alcohol was usable, but dioxan was not satisfactory as a dehydrating agent. Sections 12 to 20 microns thick were best for these studies. Phloxine and fast green F. C. F. proved most satisfactory of the several staining combinations employed.

RESULTS

Primary Invasion and Infection. Hyphae from germinated swarmspores penetrated the cuticle of inoculated fruits in a relatively short time. After a 2- to 3-hour incubation large numbers of the spores had produced appressoria and the constricted invasion hyphae arising therefrom had extended into the cuticle (Fig. 1, B). Germinating spores frequently were scattered over the entire surface area covered by the drop of inoculum. In other in-

[1] Paper No. 184 of the Scientific Journal Series of the Colorado Agricultural Experiment Station.

[2] Cooperative work conducted with Dr. L. R. Bryant, Section of Horticulture, Colorado Agricultural Experiment Station.

[3] Kreutzer, W. A., and L. R. Bryant. A method of producing an epiphytotic of tomato-fruit rot in the field. Phytopath. 34: 845–847. 1944.

[4] Sass, John E. Elements of botanical microtechnique. 222 pp. McGraw-Hill (New York). 1940.

[5] Johansen, D. A. Dehydration and infiltration. Science 82: 253–254. 1935.

stances large clusters of spores were covering and adjacent to areas where epidermal hairs apparently had been broken off (Fig. 1, A). In some cases the constricted invasion hyphae grew directly into the epidermal cells; in other cases the penetrating hypha apparently encountered a more resistant structure in the cutinized wall and grew tangentially as far as the junction of two epidermal cells. In the latter the hypha grew radially into the

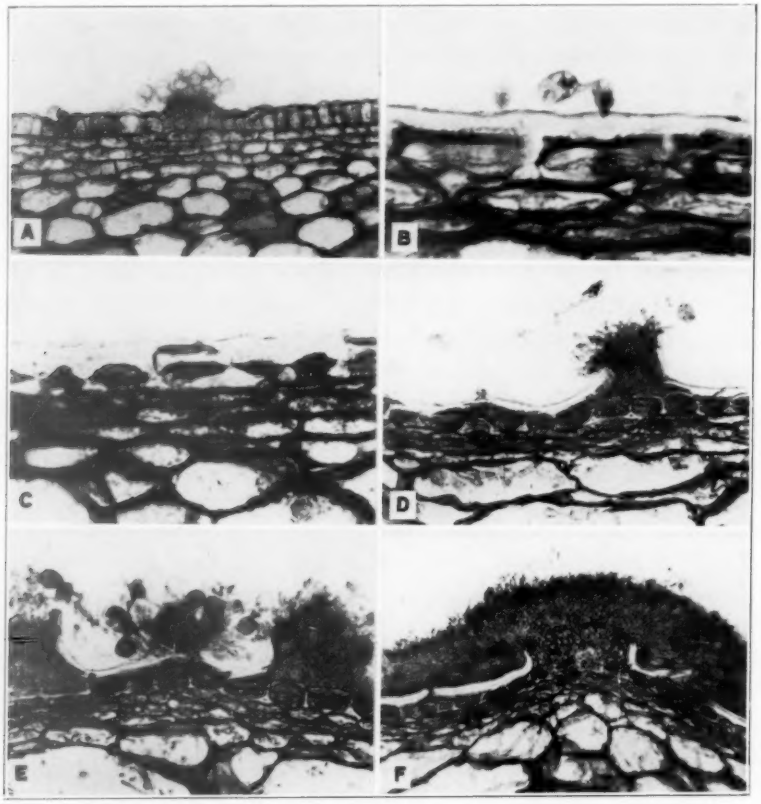

FIG. 1. Sections of tomato fruits (variety Landreth) showing invasion and fruiting of *Phytophthora capsici* Leonian. A. Swarmspores collected around a broken hair base on a green fruit, 70 min. after inoculation. B. A germinated swarmspore showing appressorium with invasion hypha entering the cuticle, 2 hr. after inoculation. C. Hypha penetrating cuticle (spore evidently removed in handling), 24 hr. after inoculation. D. Accumulated hyphae in epidermal and subepidermal cells causing rupture of the cuticle. E. Sporangia in the fungal eruption areas. F. The thick mycelial mass enclosing sporangia.

protoplast of an epidermal cell (Fig. 1, C). This condition was more frequent in tomatoes which were beginning to turn pink.

Following primary invasion, the outer tangential walls of the epidermal cells were distended by the accumulating underlying hyphae. Upon rupture of the epidermal cells and cuticle, hyphal masses emerged at points of rupture (Fig. 1, D).

Sporulation on Host Tissues. Both naturally and artificially infected fruits were examined in order to study the nature of reproduction and time required for probable sporangial formation on host tissues. Hyphal eruptions were macroscopically evident as mycelial mats of varying sizes when fruits were inoculated and held in moist chambers at 25° C. for 72 hours. Only the early stages of sporangial formation were observed in vertical sections through such areas. Fruits with the earliest indications of infection in the field had similar mycelial mats at the end of 48 or 72 hours when placed in moist chambers at 25° C. In many instances characteristic mature and immature sporangia were clearly evident in the smaller external hyphal growths (Fig. 1, E). These structures were present in the larger mycelial mats but were embedded in the hyphal matrix and consequently were not clearly visible (Fig. 1, F).

Infection Reactions in Stylar- and Stem-end Tissues. It had been observed in laboratory and field that infection of fruits occurred more readily through the stylar end than through the stem end. This difference in reaction to infection was more clearly evident in the laboratory trials because of superior controls. In general, infection occurred in 70 to 90 minutes at 25° C. in 98 per cent of the trials when swarmspores were placed on the stylar end of the tomato. In many instances where swarmspores were placed on the stem end they failed to cause typical infection areas even after 8 days incubation (Fig. 2, D and E). On the areas where the swarmspores had been placed were one to many small, sunken, rust-colored, arrested lesions without any evidence of typical watery breakdown.

In one typical trial characteristic water-soaked fruit-rot areas were evident 20 hours after inoculation as a result of all stylar-end inoculations, while there was no such breakdown from stem-end inoculations. Rust-colored arrested lesions gradually became evident on the latter inoculated fruits, and after 6 days of incubation pieces for histological examinations were taken from the affected areas (Fig. 2, E).

Except for a thicker cuticle on the epidermal cells of the stylar end, the cells of normal tissue from the two ends of the fruits were similar in structure. However, the cells of the stem-end tissue contained numerous crystals (Fig. 2, A), while no such crystals were found in tissue from the stylar end (Fig. 2, C). There were three distinct crystal types as well as certain small, non-plastid, globular masses present in the stem-end sections. Many of the crystals were six-sided, prismatic in form, and apparently of either the monoclinic or hexagonal system. Other crystals appeared cubical in cross section; many of them were elongated (Fig. 2, A) and the remainder were raphide clusters. These crystal types did not always occur in the same proportions in all fruits; sections from other tomatoes revealed a greater abundance of crystals of the raphide type.

That the crystals or some other accompanying chemical substance may have had some effect on the fungus was indicated by the fact that the hyphae which penetrated the epidermis of the stem end did not spread through the

underlying tissue as was the case when the fungus entered the stylar end. In stained sections of the arrested lesion area was an accumulation of a gum-like, reddish-brown material in the epidermal and subepidermal cells (Fig. 2, B). Often a complete breakdown of the subepidermal cells occurred in this area. Apparently the hyphal development was stopped and the characteristic watery breakdown did not result (Fig. 2, D and E).

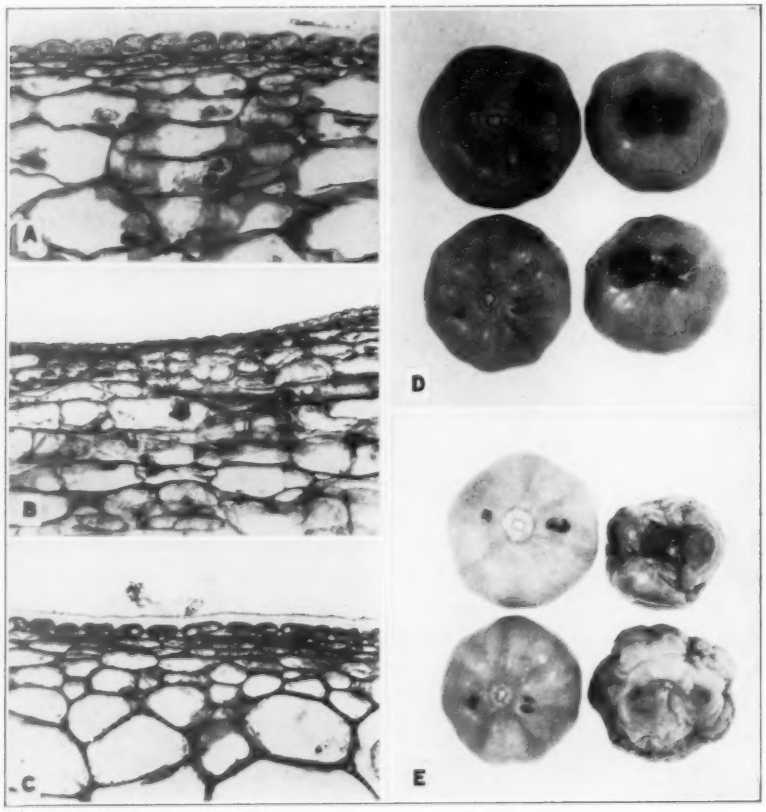

FIG. 2. Infection reactions of stylar- and stem-end tissues of tomato fruits. A. Section from stem end of Landreth tomato fruit showing presence of crystals. B. Arrested invasion in stem-end section 6 days after inoculation. Note crystals in the tissue. C. Stylar-end section showing the absence of crystals. A germinated spore is on the cuticle. D. Arrested stem-end and active stylar-end lesions in the Nebraska 642 tomato 2 days after inoculation. The variety Landreth showed the same type of reaction. E. Same fruits as in D, 8 days after inoculation.

Dufrenoy[6] observed, in cells of plants other than tomato, tannin and phenolic compounds arising as a result of the stimulus of invasion by a given pathogen. Since the bodies herein reported apparently were present in stem-end tissues of tomato fruits prior to inoculation and did not develop as a result of fungus attack, it is questionable whether they are of the same general type as those mentioned by Dufrenoy. The writers do not wish to

[6] Dufrenoy, J. Cellular immunity. Amer. Jour. Bot. 23: 70–79. 1936.

imply that the presence of these bodies in the cells of the stem-end tissues of the tomato fruits studied was directly responsible for the apparent resistance to infection. A correlation, however, existed between the presence or absence of these structures and the resistance or susceptibility of the fruit tissues tested.

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HISTO-PATHOLOGIC CHANGES IN THE PHLOEM OF AMERICAN ELM AFFECTED WITH THE VIRUS CAUSING PHLOEM NECROSIS

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INTRODUCTION

Phloem necrosis of elm was identified as a virus disease by Swingle (14) in 1938. A characteristic symptom of the disease is a yellow to brown discoloration, sometimes containing small brown to black flecks, which develops in the phloem or inner bark. In large trees, the discoloration is usually observed only in large roots and in the lower trunk. In small trees, it may occur also in the upper stem and branches. The moderately discolored tissue has a faint odor of wintergreen which cannot be detected in corresponding tissue of healthy elms. The phloem discoloration accompanied by the wintergreen odor are the only distinguishing symptoms of the disease.

As stated by Swingle (15) and as observed in inoculated 2- and 3-year-old trees cultured in soil and in a "complete" aqueous culture solution, the small fibrous roots die first. Necrosis progresses from the fibrous roots into larger roots and finally, the inner phloem in the lower portion of the stem may be killed. After the initial appearance of root symptoms, such trees usually die in two to six months. No cases of recovery have been observed. Older trees, after inoculation, may be infected for six months to one or more years before disease symptoms become apparent. Nothing is known of the duration of the "incubation period" in naturally infected trees.

The virus is readily transmitted by grafting diseased bark tissue to roots, trunk, or branches of healthy suceptible elms. Other attempts to transmit the virus by various mechanical and manual methods have failed. A natural vector of the virus has not been discovered.

Certain histo-pathological changes occur in the cells of diseased phloem tissue. This paper describes these microscopic, pathological changes in relation to the progress of the phloem necrosis from the initial infection.

REVIEW OF LITERATURE

Esau (6) summarized the anatomical changes associated with virus infections. Viruses may cause death of cells (necrosis), or an increase in size and number of cells (hypertrophy and hyperplasia), or an inhibition of growth and differentiation (hypoplasia). Two or three of these types of reactions may occur in combination. The most extensive literature on phloem degeneration pertains to the leaf-roll disease of potato reviewed by Quanjer (12) and the effects of the curly-top virus in sugar-beets and tobacco summarized by Esau (3, 4, 5, 7).

Phloem necrosis has been reported in the stripe disease of corn by Cook (1) and in the leaf-curl of raspberry by Rankin and Hockey (13). Hartzel

(8) observed hypertrophy of the phloem cells in peach trees affected by the virus causing yellows. Hyperplasia of the phloem and of the parenchyma around the phloem was observed by Magee (11) in the bunchy-top disease of banana. Lyon (10) and Kunkel (9) noted that hyperplasia and hypertrophy resulted in the formation of galls in the phloem of cane affected by the "Fiji" disease. Phloem degeneration in sugar-beets affected by curly-top is characterized by a combination of necrosis, hypertrophy and hyperplasia as noted by Esau (2, 3, 4).

EXPERIMENTAL METHODS AND RESULTS OBTAINED

To facilitate the study of root infection, inoculated 2-year-old trees were cultured with their roots suspended in a "complete," aerated, aqueous culture solution. By this method, trees could be lifted from the solution at any time and returned after examination. Inoculations were made by patch grafting diseased bark tissue to the lower portion of the stems of the healthy elms.

The first dead roots were observed 50 days following inoculation. Necrosis appeared to progress from the tips of a few rootlets. After necrosis had advanced into the larger roots, other rootlets were killed. These progressive stages of dying roots did not differ essentially from the same progressive dying observed in 2- and 3-year-old trees growing in soil where death of fibrous roots occurred one to several months after artificial inoculation.

The development and structure of the phloem tissue of the American elm has not been described previously. In the following discussion of the anatomy of the diseased tissue, reference is made to the healthy structure only where it is needed for comparison. Studies were made on the ontogeny of the root tissues of healthy American elm trees in order to be able to interpret the structure and development of comparative tissue in diseased trees.

STRUCTURE AND DEVELOPMENT OF ROOT TIPS OF DISEASED PLANTS

The general pattern of the meristematic apices of roots from diseased plants does not deviate discernibly from that of healthy roots. The arrangement of cells in the cortex and stele, and the zones of maturation in the latter, are likewise unaltered in the diseased roots. Protophloem sieve tubes are the first vascular elements that mature in the roots. In phloem tissue developing after infection, degeneration seems to follow the maturation of the primary sieve tubes. Cells immediately adjacent to these sieve tubes may undergo degeneration (Fig. 1, A and B). Usually, hypertrophy of the nuclei and cells surrounding the mature sieve tubes is the most conspicuous microscopic symptom in the primary tissues. Hypertrophied nuclei and enlarged cells are sometimes observed in the pericycle of the primary tissues. Hyperplastic cell divisions in the procambium usually follow or accompany the primary degenerative changes and continue after secondary growth

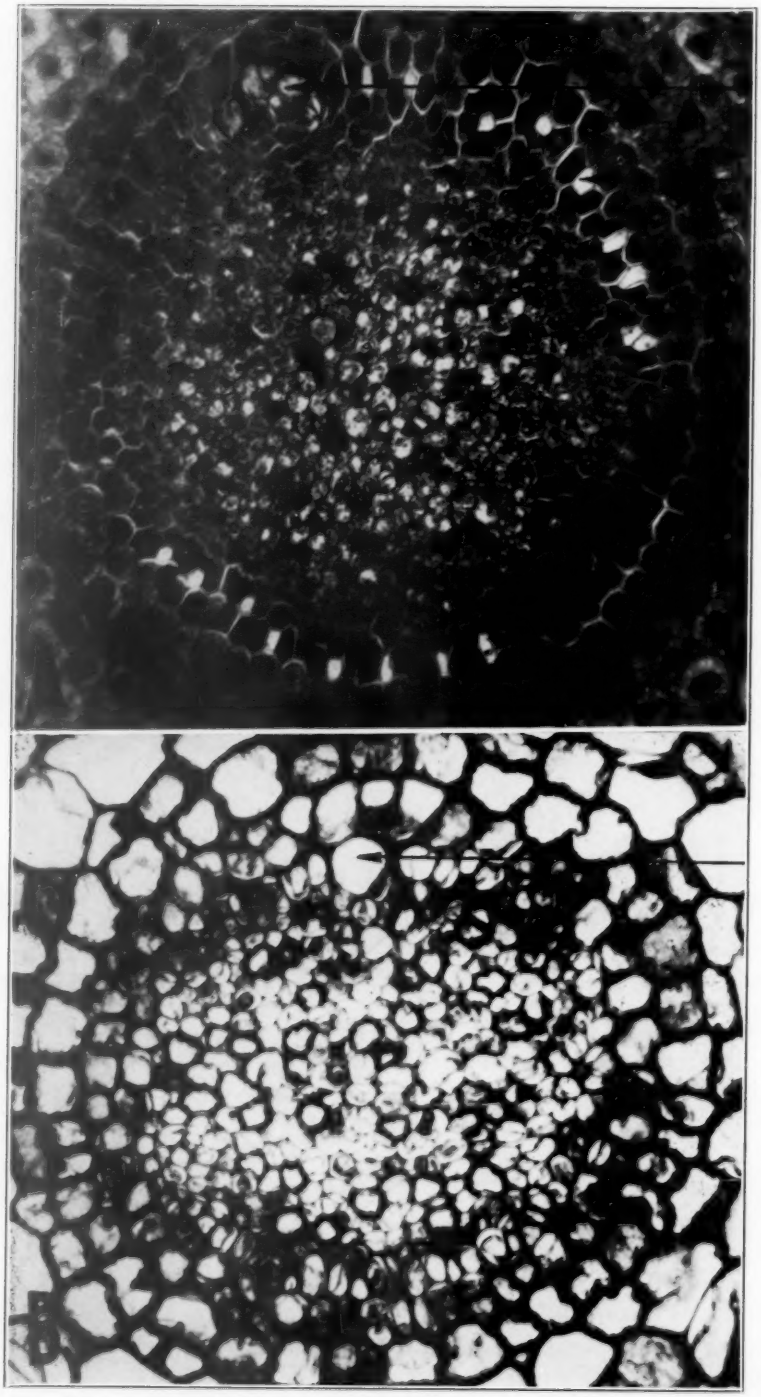

FIG. 1. Transverse sections of a root tip from a diseased American elm. The stele and part of the cortex are evident. A. Protophloem sieve tubes and hypertrophied cells adjacent to a mature sieve tube. About 700 microns from apex (×320). B. About 1200 microns from apex (×395).

begins. This symptom is conspicuous in the phloem tissue of the root or lower stem of diseased plants where the typical discoloration is apparent prior to the death of the tree (Figs. 2 and 3). Certain cells of the protophloem may undergo necrosis. Before vacuolation of cells in the regions of the primary phloem mother cells, certain of the isodiametric cells stain very darkly. These cells appear to be immature sieve-tube cells. In older roots, some of the sieve-tube cells undergo degeneration, which may indicate that the sieve tubes are primarily concerned in virus transport as well as being the seat of necrosis. These necrotic cells are usually compressed by adjacent parenchymatous cells.

STRUCTURE OF SECONDARY TISSUES IN HEALTHY AND DISEASED ROOTS

Secondary thickening of the root results from cambial activity which occurs in the zone of fundamental parenchyma lying between the central metaxylem vessels and the primary phloem. Secondary xylem consists of large vessels, smaller conductive cells, fibers and parenchyma. The parenchyma appears as rays that are from one to several cells in width, the cells being radially elongated. The large vessels form in radial rows typical of spring and summer growth, but become somewhat irregular in arrangement in the older annual rings owing to the increase in their size. The typical diarch protoxylem retained its identity in the center of the woody cylinder in roots up to 3 years old in the preparations studied.

Outside of the cambial zone, the secondary phloem has a layered appearance which results from the differentiation of zones of small, thick-walled fibers, that alternate with regions of parenchyma, sieve tubes and companion cells. The sieve tubes lose their identity in the outer phloem and only the fibers and parenchyma cells are conspicuous. Parenchymatous ray cells in the phloem are continuous with the xylem rays. The ray cells are apparently of cambial origin. As the root increases in size, the cortical cells and epidermal cells are stretched and ultimately disintegrate. Distortion of the cortical tissue in diseased material due to the virus infection has not been observed. During the secondary phases of growth, the pericycle remains active and a phellogen develops from it. The bark tissue of older roots, following the normal disintegration of the cortical tissue, consists of phloem tissue derived from the primary cambium with an outer corky periderm.

The same developmental pattern is characteristic in the diseased as in the healthy root. Xylem of diseased plants does not differ from that of healthy plants. After infection, the recently developed phloem next to the cambium zone has no regularity in cell differentiation (Fig. 2, A). Degenerative changes are more noticeable in the tissue developed subsequent to infection.

The most striking characteristic of older diseased phloem tissue of both root and stem under microscopic observation is the almost complete destruction of sieve-tube cells, with a marked increase in number and size of the parenchyma cells. Some cells divide in any plane, others enlarge without

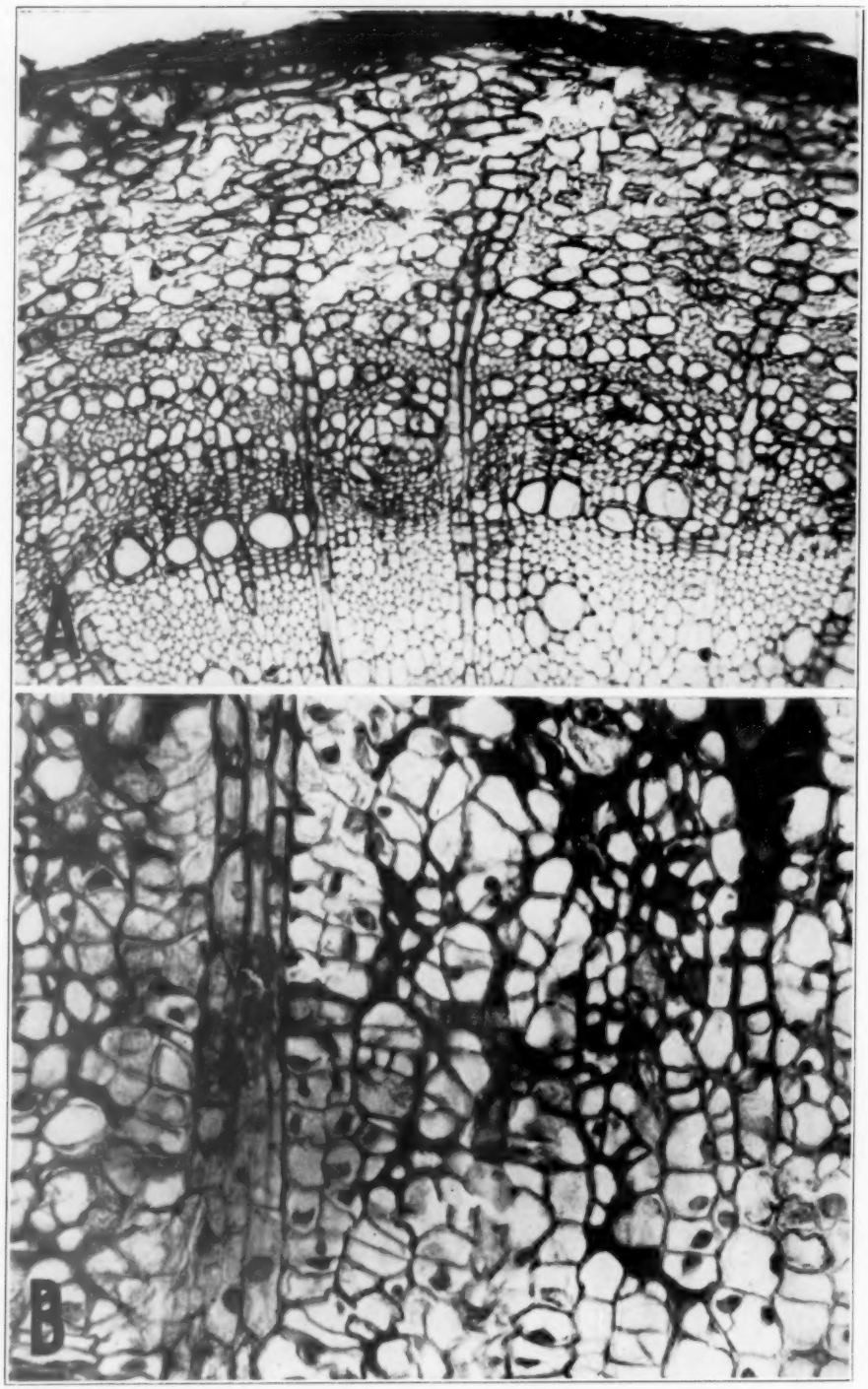

FIG. 2. A. Phloem and part of the xylem as seen in transverse sections of a diseased 3-year-old American elm root (×117). B. Necrosis, hypertrophy and hyperplasia in a transverse section of diseased phloem tissue from stem (×435).

dividing, and their contents disintegrate. In later stages, certain cells collapse, resulting in the formation of cavities which are filled with degenerate cell material and a yellowish, dark-staining, granular substance (Figs. 2 and 3).

Occasionally, the yellowish substance becomes concentrated immediately adjacent to the cell walls, resulting in irregular thickenings. Usually, it is dispersed throughout the lumina of certain phloem cells and dissolves out in fixation. Preliminary microchemical studies indicated that this material has certain properties of suberin. It seems to be anisotropic and stains yellowish-red with Sudan III.

Sieve tubes are difficult to locate in prepared sections of the hyperplastic tissue. In transverse sections, they are of about the same size as unaltered parenchyma cells, have transverse sieve plates, and are usually surrounded by abundant thin-walled parenchyma. The lateral walls of sieve-tube cells, which abut upon other sieve tubes in the secondary phloem, are frequently covered with pronounced lattices. The affected cells are crushed following enlargement and excessive divisions of the surrounding cells. The degenerated cells are destroyed and the yellowish, dark staining material may run between adjacent parenchyma cells and fuse with that of other degenerated cells (Figs. 2 and 3).

Structure of secondary tissue in healthy and diseased stems

The structure of the woody cylinder of the stem has essentially the same pattern as the woody cylinder of the root. The xylem of the stem is ring porous and is transversed by numerous parenchymatous rays which have a common union surrounding the centrally located pith. Growth rings can be distinguished in the xylem owing to somewhat smaller and more compactly arranged vessels at the outer limit of each ring. The xylem tissues in diseased plants do not differ from comparable tissues in healthy plants.

Secondary phloem consists of sieve tubes, companion cells, fibers, and parenchyma. It has a banded appearance, much the same as the older phloem tissue of the roots, owing to the differentiation of tangential bands of fibers which form the outermost portion of each growth zone. The intervening bands of sieve tubes, companion cells and parenchyma may become crushed in the natural growth processes and increase in diameter of the stem. A phellogen layer, from which a periderm develops, differentiates in the cortex beneath the epidermis. During successive growth periods, the phellogen differentiates deeper in the cortex and finally in older stems the cortex is sloughed off and the bark consists entirely of phloem tissue with a corky periderm and has much the same structure as the bark of older roots. As in the roots, sieve tubes are difficult to distinguish in the older phloem tissue of stems.

The structure of diseased phloem in the stem is similar to that in diseased roots, except that hyperplasia seems to be more noticeable (Figs. 2, B, and 3). This may be explained from the fact that roots die first, while the

degeneration in the phloem of the stem may be carried over a longer period of time.

In fresh sections of bark tissue cut from a diseased tree, the yellow to brown discoloration, associated with a faint wintergreen odor, distinctly marks the boundary of the current phloem development. Observed macroscopically, it does not appear to extend into the older phloem tissue, and has never been observed in the xylem. Occasionally a yellow to brown dark-staining amorphous material is present in diseased cells. This material may be finely granular or consist of irregular globular masses. It is not known what relation this material has to the presence of virus in the discolored

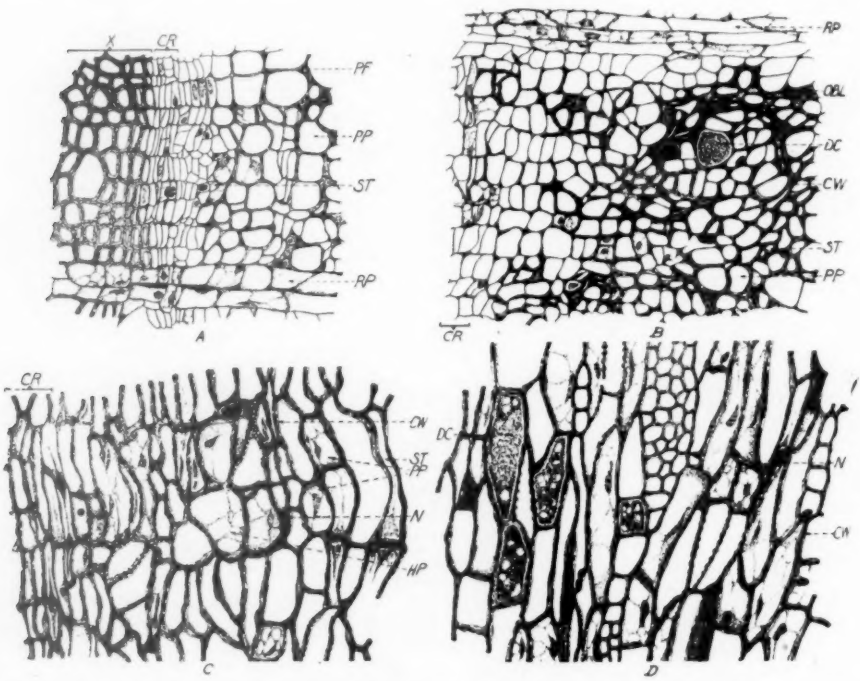

FIG. 3. Diagrammatic sketches of diseased and healthy phloem tissue from American elm stems. A. Transverse section of healthy phloem tissue. B, C, D. Transverse, radial and tangential sections, respectively, of diseased phloem tissue. Details are: CR, cambium region; DC, diseased cells; PP, phloem parenchyma; PF, phloem fibers; HP, hypertrophied phloem cell; CW, enlarged cell wall; RP, ray parenchyma; X, xylem tissue.

tissues. It is not certain whether necrosis is primary or secondary to the accumulation of the material. The discoloration is first observable in the phloem tissue of the roots. Later it may be observed in the lower trunk, sometimes being more pronounced on one side of the tree than on another. In an advanced stage of root necrosis the phloem tissue of the lower stem may have a typical dark discoloration which shades into a lighter color as it extends into the lower branches. Microscopic examinations of stained sections of twigs without discolored phloem, but taken from trees in which the discoloration was pronounced at the base revealed no distinguishing patho-

logic changes. Likewise, microscopic preparations of leaf sections taken from infected trees had no observable pathologic changes.

The discoloration seems to be due to a substance which becomes concentrated during phloem necrosis in the roots and it progresses into adjacent phloem tissue preceding degeneration and necrosis, or is formed coordinately with the destruction of tissues. It is not known whether this material is a product of the disturbed physiological processes of the diseased cells, whether it is material from the degenerated necrotic cells, or whether it might be associated with the virus entity itself. In severely discolored tissue, it closely resembles wound gum.

It is difficult to account for the wintergreen odor associated with diseased tissue, but it is apparently associated with the virus infection since the odor cannot be detected in healthy elms.

SUMMARY

Phloem necrosis is a virus disease of elm. The virus is readily transmitted by grafting diseased bark tissue to healthy suscepts. A natural vector has not been discovered.

Following infection, the fibrous roots die first. Necrosis progresses from the fibrous roots into the larger roots and finally, after death of the roots, the inner phloem in the lower portion of the stem may be killed.

Microscopic pathological changes in the primary phloem tissue of the root include hypertrophied cells and nuclei in the vicinity of mature sieve tubes, followed by hyperplasia and finally crushing of the sieve-tube cells and companion cells in the older tissues. Hyperplasia and hypertrophy of parenchyma are the most striking microscopic symptoms in the phloem of older roots and stem.

A yellow to brown discoloration of the phloem tissue accompanies the death of the roots and usually progresses into the stem and lower branches before the tree dies. Associated with the discoloration is a faint odor of wintergreen in freshly cut tissues, which is not detectable in comparable healthy tissues.

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HOST–PARASITE RELATIONS IN RED ROT OF SUGAR CANE

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Information on how a fungus grows through the tissues of its host, how it penetrates the epidermal layers, how it again breaks out to the surface to produce its spores, and how the host reacts to the advancing mycelium is necessary to understand the disease which it causes. Cell walls, some of which are of considerable thickness, are penetrated and the protoplasm and other cell contents are changed to a greater or less extent as the fungus absorbs the nutrients essential for its own growth.

In recent years, botanists have shown considerable interest as to how cell-wall penetration takes place and there have been various theories to explain it. The usual explanations have been either that the fungus produces an enzyme which dissolves the cellulose in advance of the invasion thread or that the fungus actually exerts enough pressure to force the hypha through the cellulose wall. In the more recent literature the latter theory is perhaps the more generally accepted.

The reaction of the protoplasm to the advancing mycelium involves many complex biological and chemical problems. This concerns the questions of resistance and susceptibility and must in the future receive more consideration than it has in the past.

For many years while studying the red rot of sugar cane caused by the fungus *Colletotrichum falcatum*, now known as *Physalospora tucumanensis*, numerous sections of diseased sugar-cane tissues have been examined and it has been possible to observe the cell-wall penetration by the mycelium and to watch the changes in the protoplasm. While it is not possible to present definite evidence to support either of the theories of cell-wall penetration, or to explain the protoplasmic changes observed, much information has been obtained on what actually occurs.

Sugar cane is an extremely useful and valuable plant for studying host-parasite relationships. The plant is large and the elements in the stem and leaf are unusually well separated. The parenchyma cells in which the sucrose is stored are large and the cell walls are firm, relatively thick, and have numerous pits which are large enough to be plainly seen in ordinary freehand sections. Furthermore, sugar cane is propagated vegetatively. It has long been recognized that this type of propagation accounts to a very large extent for the severe and very devastating disease epiphytotics which have occurred on sugar cane in the past in various parts of the world. All plants of a variety, being identical from a genetic standpoint, are equally susceptible to any new parasite or any new parasitic strain of an organism when conditions are favorable for the plants to be attacked. The occurrence of such an organism and such conditions have in the past meant crop failures.

Such a plant, however, is ideal for various physiological, histological, and pathological studies.

REACTION OF SUGAR-CANE TISSUES TO RED ROT

The tissues of the sugar-cane plant react variously to the presence of the red-rot fungus. Mycelium from spores germinating in the ducts of the fibrovascular bundles or from other centers of infection grows out very rapidly through a number of layers of cells. While a certain amount of variation occurs in the growth of this mycelium, the invaded cells ordinarily do not immediately become filled with mycelium, as the mycelial threads after entering a cell usually branch but little and very commonly grow directly across the cell to the opposite wall and into the adjoining cell.

Eventually, however, there is a reaction or a change of some kind in the host cells in advance of the invading mycelium. The protoplasm changes in color and a gummy dark red material oozes out of the cells and fills the intercellular spaces (Fig. 1, C, D). It is not clear whether these modifications are merely due to the disintegrating protoplasm or not. This zone in advance of the mycelium in which the changes occur turns red, because of the presence of a soluble dye which is absorbed by the cell walls (Fig. 1, B). Under some conditions, especially in standing cane, the fibrovascular bundles may become red several inches from the center of infection. The growth of the advancing mycelium is stopped or at least checked temporarily by this red zone. Whether this is due to the presence of some counteracting toxic substance, to the plugging of the pits in the cell walls by the gummy material, or for some other reason is not clear.

When this zone develops there is then present the typical or characteristic lesion of red rot, a white or straw-colored spot with mycelium surrounded by a distinct red border with little or no mycelium. How large this lesion becomes depends largely on the natural resistance of the variety and the condition of the stalk (Fig. 1, A). The natural resistance of a variety seems to depend on how rapidly the counteracting changes occur, or, in other words, how soon the red zone around the infected area develops. In standing cane of resistant varieties, the lesions may remain very small, while in susceptible varieties they may extend entirely across the stalk. The reaction of the cane tissues, especially in resistant varieties, may be so rapid that the first invaded cells take on the red color. When this happens the lesion remains very small and often the reaction is so great that the invading mycelium is killed before it has a chance to spread materially.

The intensity of the reaction of the cane tissues is also influenced by factors which affect the functioning of the cells. In stalks which are not functioning normally, as for example stalks which have been cut and placed in a moist place, the response of the host cells to the invading mycelium is not so pronounced and often even in resistant varieties the growth of the mycelium is not greatly restricted. The interior of such stalks may be mottled but the red zones are rather indefinite and even the fibrovascular

bundles often fail to absorb any red dye. In cut stalks which have been placed in a warm situation and especially if allowed to dry to a certain extent, there is very little if any response of the host cells to the fungus. The whole interior of such stalks becomes permeated with mycelium and areas of various sizes become black in color. In these areas the fungus grows

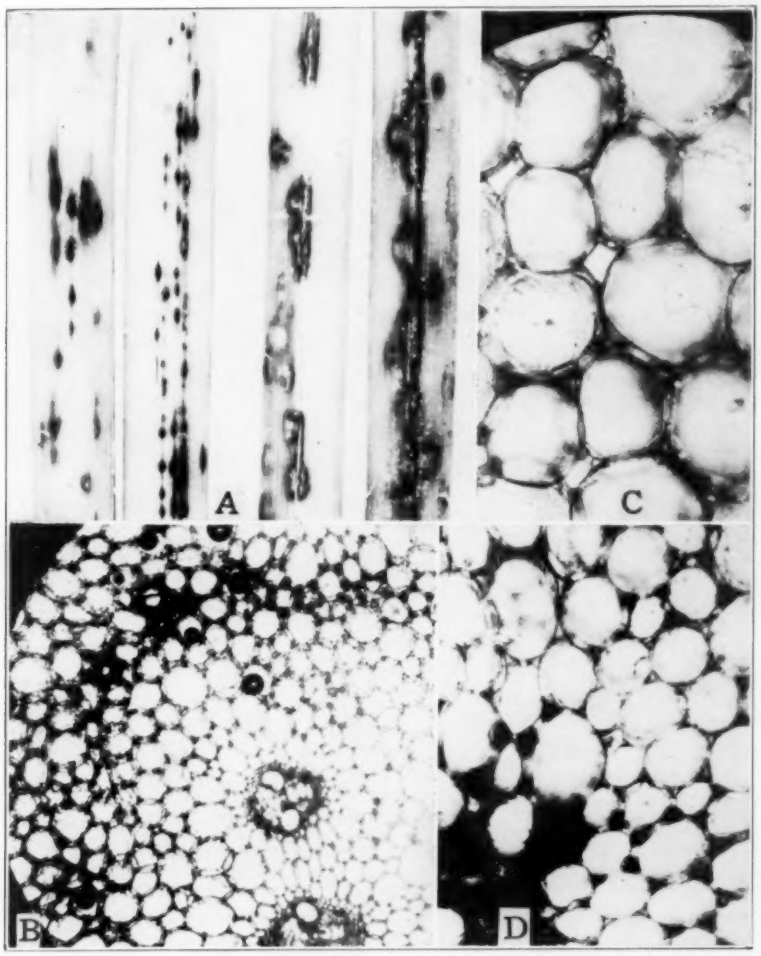

FIG. 1. Lesions of red rot in interior of cane stalk. A. Lesions as they appear in split stalks: on left, a resistant variety, Co. 281; on right, a very susceptible discarded seedling. B. Cross section of stalk through a lesion, red zone above and to the left. C. Early stage of red zone showing droplets of gummy material oozing out into the intercellular spaces. D. Later stage showing intercellular spaces filled with gummy material.

unrestricted, as it does in culture media, and the cells become filled with large hyphae which are brown in color.

The mycelium of *Colletotrichum falcatum*, then, in tissues of sugar cane varies from the relatively small and scattered hyaline threads in the newly infected regions to the network of thick, brown threads found in the old

dead areas. While the rate and extent of the growth of this mycelium depends to a certain extent on the natural resistance of the variety, this resistance can be modified and broken down by factors affecting the functioning of the host cells.

PENETRATION OF CELL WALLS IN LESIONS BY HYPHAE

Apparently in all cases in the internal parenchyma the mycelium passes from cell to cell through the pits in the cell walls. In the young, active hyphae the hyphal tip is small and swelling of the mycelium on opposite

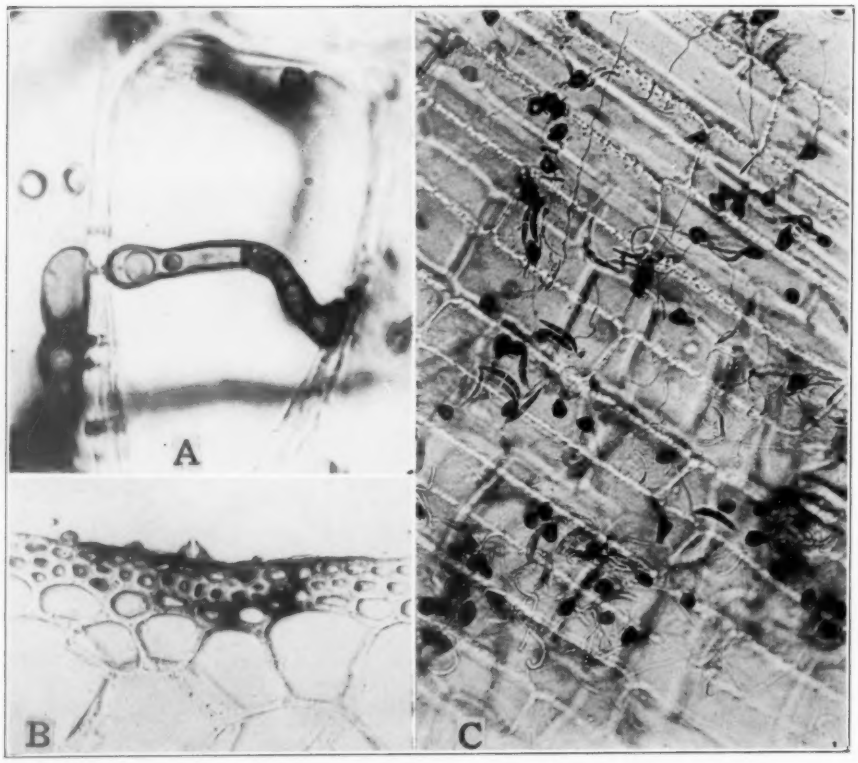

FIG. 2. A. Mycelium passing through pit in an old stalk lesion, variety C.P. 34/79. B. Cross section of red lesion on upper surface of midrib below appressoria, 17 days after inoculation, variety C.P. 34/120. C. Appressoria on inner surface of leaf sheath, variety C.P. 34/120.

sides of the cell wall where it passes through a pit is not pronounced. On the other hand, the old, brown hyphae within the cells in the black lesions are much thicker than the small segments passing through the pits. Frequently with such the hyphal thread is swollen on each side of the cross wall (Fig. 2, A). Whether this swelling takes place before the penetration of the pit has not been determined. There is, however, no evidence to indicate that there is any enlargement of the pit by any enzyme which might destroy or erode some of the cell-wall cellulose.

INFECTION THREADS FROM APPRESSORIA

Conidia of the red-rot fungus, when placed on the uninjured epidermis of the leaf or stem, germinate and produce single-celled, brown appressoria which become cemented to the substrate on which they develop. With related fungi it is known that appressoria produce germ tubes which penetrate the host tissues by going directly through the cell walls. Such a method of infection has been suggested for the red-rot fungus, but, in the past, inoculation experiments made by placing spore suspensions on the uninjured epidermis of the cane plant have in general not been convincing, and infection, except through wounds, has not been demonstrated with certainty.

To determine definitely whether the appressoria function in infection, and if so to determine how the infection occurs, a number of tests were made during the summer of 1943. Spore suspensions were sprayed on various parts of the cane plant. In spite of the fact that the weather was unusually dry while the tests were made, the conidia germinated readily and quickly produced appressoria (Fig. 2, C). The inoculated areas were examined over a period of several days for the development of lesions and numerous freehand sections were examined for the presence of infection threads from the appressoria.

In some of the inoculation tests, spore suspensions were sprayed on the upper surface of midribs. In general, satisfactory infection was not obtained in these tests. A few small lesions were observed but these did not increase in size. In sections through these lesions (Fig. 2, B) the walls of 3 or 4 layers of cells were reddened, but mycelium was not found with certainty in the cells and no infection threads were found in the thickened walls of the epidermal cells.

In other tests the spore suspensions were dropped in behind the leaf sheaths of young active leaves and allowed to come in contact with the inner epidermis of the leaf sheath and the young growing stem. The epidermal layers of these structures were tender and the cell walls were soft. On such surfaces, infection was obtained easily and rapidly. In 3 to 4 days lesions extending entirely through the leaf sheath were observed. Sometimes the whole leaf sheath later became discolored and large dead spots developed (Fig. 3, A, B). Also, numerous small lesions formed on the young stems (Fig. 3, C). While these stem lesions turned red and often penetrated 2 to 4 layers of cells, they were not observed to penetrate through the dense fibrovascular layer to the interior of the stem. In the lesions on the leaf sheaths and stems, acervuli with spores developed quickly. The infection on the leaf sheaths was similar to that which sometimes occurs in nature. Ordinarily, in the field, spores developing in acervuli on the midrib are washed down to the ligular region and, when possible, behind the leaf sheath. That more natural infection does not occur on leaf sheaths in the field is apparently due to the fact that the coating of wax makes a watertight seal between stalk and leaf sheath.

In the sections made from recently inoculated leaf sheaths, infection threads were found. These were sent out from the contact surface of the appressoria and penetrated the cell walls of the epidermis (Fig. 4). These threads were extremely small and penetrated the wall usually in a straight line and generally either perpendicular to the leaf surface or parallel to the cross walls of the subepidermal layer. In no case was any erosion observed in the cellulose wall around the infection thread, and consequently there was

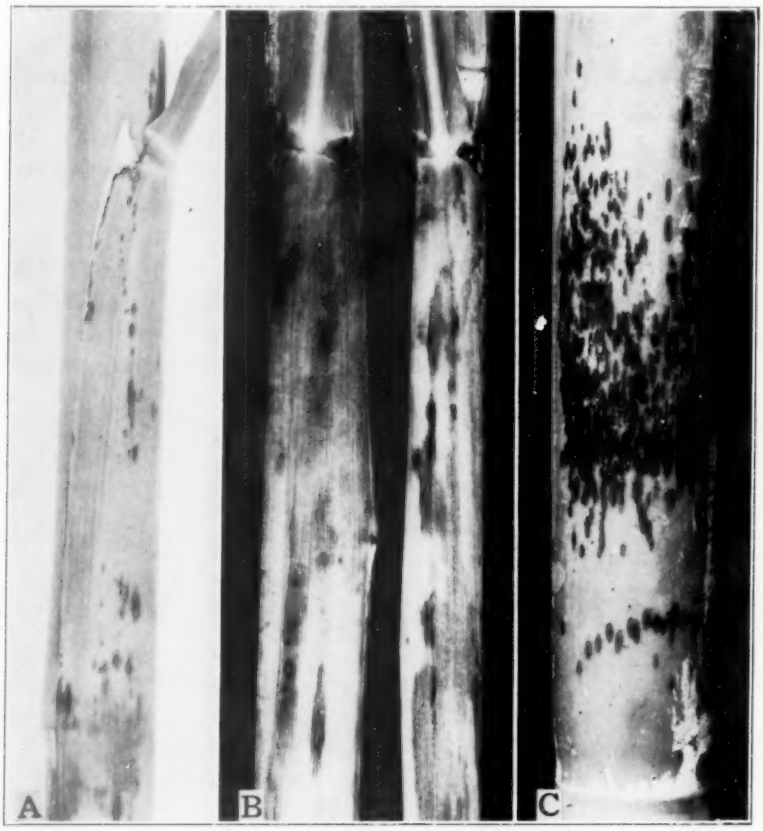

FIG. 3. Red-rot lesions from spore suspension dropped behind leaf sheath, variety C.P. 34/120. A. Leaf-sheath lesions, 10 days after inoculation. B. Leaf-sheath lesions 15 days after inoculation. C. Lesions on young stalk, 5 days after inoculation.

no evidence suggesting that the thread went through a pore dissolved out by an enzyme. These infection threads, which were easily seen when stained with cotton blue, were often quite numerous.

How the threads penetrated the wall was not determined. Pits were not observed in the outside walls of the epidermal cells. It does not seem reasonable that an extremely delicate thread could penetrate a solid cell wall by pressure without some twisting and bending. The demonstration of pores, pits, or at least lines of cleavage perpendicular to the surface, would be extremely helpful in explaining these straight infection threads.

The 2 walls of the epidermal cell on the inside of the leaf sheath are very close together, often being almost in contact. Very frequently the infection thread penetrated both walls with no apparent widening out in the very narrow lumen.

After entering a cell of the second layer, the infection threads widened out to the normal small type of hyphae. These hyphae, however, continued to grow directly towards the center of the leaf sheath, often elongating parallel to the cross walls of the cells (Fig. 4, E). Often a somewhat flattened body was visible where the infection thread first entered the interior of some of these cells. These, however, were not always present and their

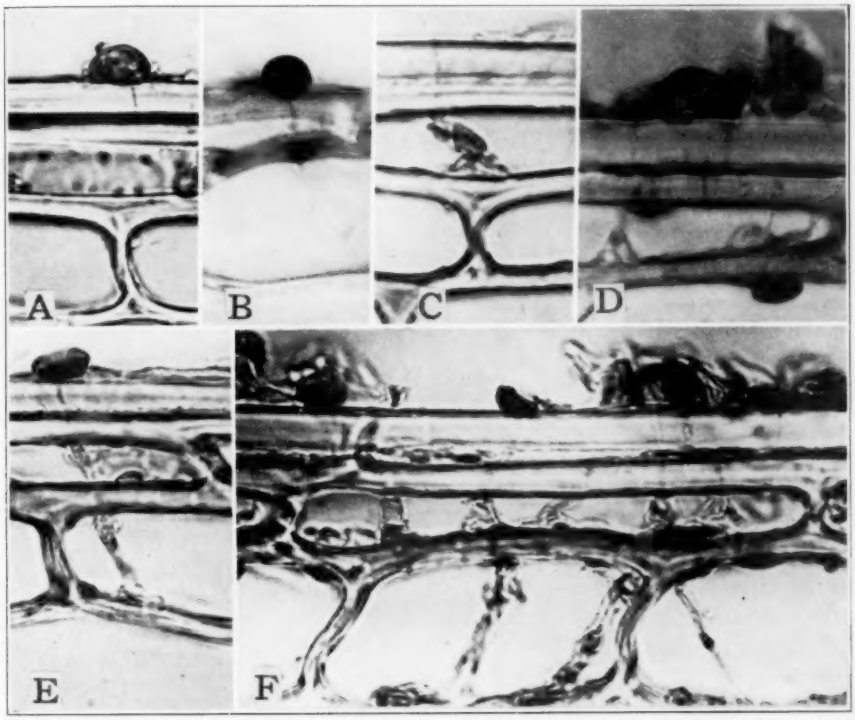

FIG. 4. Infection threads and mycelium from appressoria, variety C.P. 34/120. × 780. A. Early stage of infection thread. B. Infection thread 4 days after inoculation. C. Infection thread connected to mycelium in subepidermal cell. D. Appressoria with infection threads, mycelium, and black body. E. Infection thread and mycelium growing toward center of leaf sheath but parallel to cell wall. F. Many infection threads, several connected with mycelium.

nature was not determined. No enlargement of the hypha on opposite sides of the cell wall was observed in these newly infected cells.

In infection from the appressoria there was no indication that mycelium from an infection thread after passing through the cuticle becomes dormant between the cuticle and the cell walls of the outer layer of the epidermis. Such has been shown by Simmonds[1] for some of the anthracnoses found on

[1] Simmonds, J. H. Latent infection in tropical fruits discussed in relation to the part played by species of Gloeosporium and Colletotrichum. Proc. Royal Society of Queensland for 1940 52(2): 92–120. 1941.

mango, banana, and other tropical fruits. Whether such a condition ever occurs in the leaf blade of sugar cane has not been determined. It is known, however, that the red-rot fungus often will break out all over a leaf placed in a moist chamber, and this is characteristic of a dormant stage of a parasite.

ACERVULUS FORMATION

After growing for a period within the tissues, the red-rot fungus breaks out to the surface and produces its spores in an acervulus. Whether the

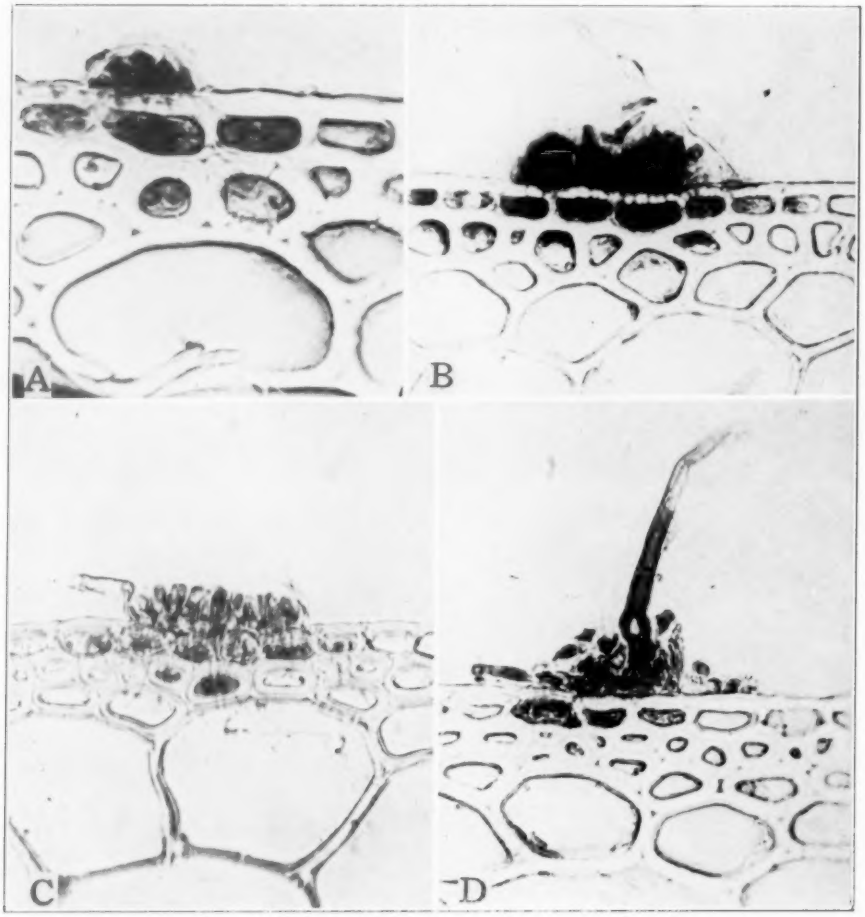

FIG. 5. Development of acervulus between cell wall and cuticle on upper surface of midrib, deep-staining mycelium in epidermal cells and fine threads through cell wall, cross section, variety C.P. 34/120. A. Young acervulus with cuticle still unbroken. ×780. B, C. Older lesions with cuticle ruptured. ×390. D. Still older stage with setae. ×390.

acervulus is a structure sufficiently developed and differentiated to be considered a fruiting body is open to argument. It is in fact nothing but a cluster of conidia-bearing conidiophores intermixed with a number of long, dark-colored setae. Both the conidiophores and setae develop directly from the mycelium in the tissues.

Acervuli are ordinarily very abundant on both surfaces of the midrib. These develop quickly and as there is very little breakdown of the midrib tissues, especially of the upper epidermis, the formation of the acervuli can be readily followed by a study of free-hand sections.

The thick, hard, membranous epidermis of the upper surface of the midrib consists ordinarily of about 3 layers of thick-walled cells which are

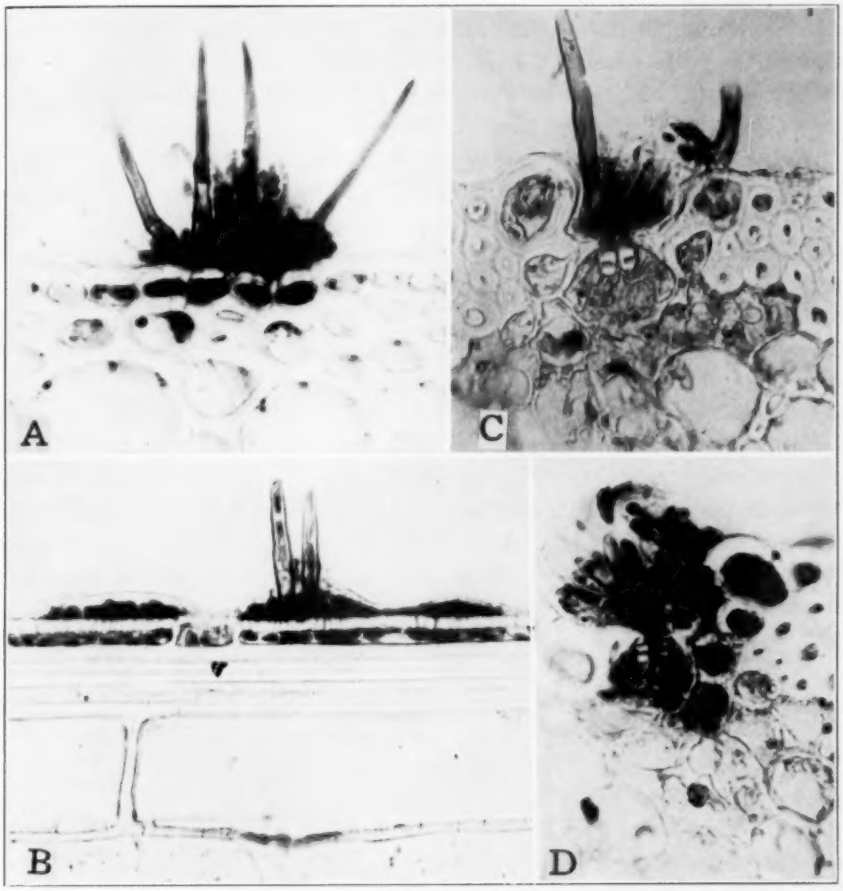

FIG. 6. Acervuli, variety C.P. 34/120. ×390. A. Old acervulus with spores, upper surface of midrib, cross section. B. Acervulus, upper surface of midrib, longitudinal section. C, D. Acervuli from stomates, under surface of midrib, showing deeply staining mycelium in epidermal cells and fine threads through cell walls.

elongated in the direction of the main axis of the leaf. In the formation of the acervuli (Figs. 5, 6), these cells and especially the ones in the outside layer, become packed with mycelium. This mycelium is very active and apparently contains much nutritive material, as it stains much more deeply with cotton blue than do the hyphae found in the interior tissues. Very small hyphae then pass through the outside cell wall from this mycelium.

This is the reverse of what occurs in infection from appressoria. Why these small threads pass inward through the cell wall from the appressoria and outward from the active hyphae in the epidermal cells is another of the unexplained host-parasite relationships. After passing the cell wall, the small threads immediately begin producing the conidiophores and setae between the wall and the cuticle (Fig. 5, A). Apparently the nutritive material necessary for spore formation comes through the fine threads from the active mycelium in the epidermal layers. As the conidiophores and setae develop, the cuticle separates from the cell wall and is pushed up until it finally ruptures (Fig. 5, B, C). This subcuticular development is characteristic of acervulus formation on the upper surface of the midrib.

On the under surface of the midrib, the mycelium, taking the easiest way, pushes out through the stomates (Fig. 6, C, D). But even here the acervulus is reinforced by mycelium which is packed in the thick-walled cells bordering the channel in which the stomates are located. Small threads pass out from these cells and produce conidiophores just as they do from the cells on the upper surface of the midrib.

Acervuli also form when only a single layer of cells is infected and even form on single-celled plant hairs which contain mycelium.

The fine threads passing through the cell walls and supplying nutrients to conidiophores for the production of conidia, in contrast to the infection threads which develop from the appressoria, function for days or even weeks. If these threads produce an enzyme which may act on the cellulose, its action should be evident. Sometimes, in sections of rather old acervuli, there has seemed to be some erosion of the cellulose around the fine threads and in a few cases it appeared as if the acervulus had broken back into the outer layer of epidermal cells but the latter cases were not common.

SUMMARY

A study was made of some of the host-parasite relationships of the red rot of sugar cane, including cell-wall penetration and the reaction of the host cells to the invading mycelium.

An internal lesion of red rot typically consists of a white to straw-colored center surrounded by a red zone.

In advance of the mycelium the protoplasm of the host cells becomes modified and a gummy material oozes out and fills the intercellular spaces. At the same time, a reddish dye-like substance is produced which is absorbed by the cell walls.

Mycelium advances but slowly into the red zone. The red zone forms quickly in resistant varieties and more slowly in susceptible ones.

In stalks in which the cells are not functioning normally, the mycelium spreads more readily than in standing cane.

In the stalk and leaf, the mycelium penetrates the cell walls through pits.

Infection threads from appressoria were observed in the epidermal cell walls of the leaf sheath.

Acervuli develop from mycelium packed in the epidermal and subepidermal layers. This mycelium sends very small threads through the walls of the epidermal cells. The conidiophores and setae develop from these threads. The acervuli are subcuticular.

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SEEDLING INFECTION OF DENT MAIZE BY SCLEROTIUM BATATICOLA TAUB.[1]

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(Accepted for publication May 10, 1944)

INTRODUCTION

Sclerotium bataticola Taub. causes a seedling disease of many crop plants in the field and under greenhouse and laboratory conditions. Its attack on corn, as generally observed, is confined to the mature or nearly mature plants. Its parasitism on seedlings, here reported, has not been noted previously.

The charcoal-rot disease of dent maize was found in Iowa in 1941 for the first time[2] and was found again in 1943. In 1941, the disease occurred in mid-August, in only trace amounts in one field near Ames. The corn in this field was planted early and was ripening prematurely with approximately 30 per cent of the stalks dead in mid-August. The soil was Webster silty clay loam. July and August were very dry and the corn throughout the state ripened somewhat earlier than normal, particularly on the knolls where drought conditions were more severe.

In 1943, the disease was found in mid-September on approximately 33 per cent of the plants in parts of several fields in a small area, approximately 2 miles south of Cherokee, Iowa. The plants in these fields were nearly all dead or dying, approximately 50 per cent of them being broken over several nodes above the ground. The corn outside this area and throughout the state was still very green and upright. Rainfall was above normal throughout most of the State in 1943 except in the area indicated, where the rainfall was much below normal during August and September. In addition, the soil in this area was Dickinson loam having light texture with sandy to gravelly subsoil.

METHODS

Corn-seedling infection was studied in the greenhouse during the winter of 1941–42. A culture of *Sclerotium bataticola* was used which was isolated in mid-August, 1941, from the stalk of a diseased corn plant in a field near Ames, Iowa. Inoculum was prepared by growing the fungus for 3–4 weeks on 50 gm. of a 5 per cent corn-meal-soil mixture contained in 125-ml. Erlenmeyer flasks and wetted to approximately 70 per cent of its water-holding capacity. Weighed amounts of this inoculum were added to each 4-inch pot of soil for the infested series, while no additions were made to the non-infested series. The inoculum was distributed uniformly as a thin layer at

[1] Journal paper No. J-1187 of the Iowa Agricultural Experiment Station, Ames, Iowa, Botany and Plant Pathology Section, Project No. 577.

[2] Semeniuk, G. Charcoal-rot of maize, new to Iowa. (Abstr.) Proc. Iowa Acad. Sci. 49: 256. 1942.

the seed level or at some measured distance above or below it. Seven seeds were planted in each pot and usually 3 weeks were allowed for seedling development.

The soil used was 2 parts greenhouse compost to one part of washed river sand, steamed for 5 hours at 15 lb. pressure and used immediately. Non-disinfested seed of inbred line Hy was used in all the tests.

The developed seedlings were washed out and measured for: wet weights of seedlings per pot; height of individual seedlings; mesocotyl and primary root necrosis of individul seedlings. The mesocotyls were rated 0, 1, 2 or 3 for disease development indicating that they were healthy, a trace to $\frac{1}{3}$ necrotic, $\frac{1}{3}$ to $\frac{2}{3}$ necrotic, or $\frac{2}{3}$ to completely necrotic, respectively. The primary roots (radicle and seminals) were rated 0, 1, 2 or 3, indicating that there was no necrosis, one to several small necrotic areas on one or more roots, several elongated necrotic areas usually on two or more roots, much elongated necrotic areas up to complete or nearly complete necrosis of one (radicle) but usually all members of the primary root system, respectively. The disease severity for a group of seedlings was determined by the following formula:

$$\frac{\text{Sum of ratings given individual plants} \times 100}{\text{Number of plants examined} \times 3}.$$

DISCUSSION OF RESULTS

No pre-emergence rotting of seed occurred in *S. bataticola*-infested steamed soil. The seedlings developed normally for approximately 2 weeks, whereupon stunting and reduction in vigor of the seedlings became manifest and later accentuated (Fig. 1, A). Not all the seedlings in any one pot infested with *S. bataticola* were similarly affected. A few seedlings remained as vigorous as those in the non-infested pots of soil. In general, the occurrence and the extent of stunting reflected the progress of mesocotyl and primary root necrosis.

In most of the seedlings in the infested soil, necrosis was severe in mesocotyl and primary root. In the milder cases of necrosis the affected tissues were light brown and without sclerotia of *S. bataticola*, while in the more severe cases they were dark brown to near black because of an abundance of sclerotia (Fig. 1, B). The discoloration probably was due to other organisms accompanying *S. bataticola*, particularly *Fusarium moniliforme*, since many infected roots bearing an abundance of sclerotia were colorless, while discolored tissues frequently yielded *F. moniliforme* and other organisms along with *Sclerotium bataticola*. When mesocotyl necrosis was severe, the tissues were partially disintegrated and easily torn apart by the force of fine streams of water during washing. In severe necrosis of the seminal root the cortex was completely disintegrated and absent, leaving intact only the stele and epidermis.

Although sclerotia frequently were on the pericarp, mesocotyl, coleoptile, primary and occasionally secondary roots (Fig. 1, B), their absence in

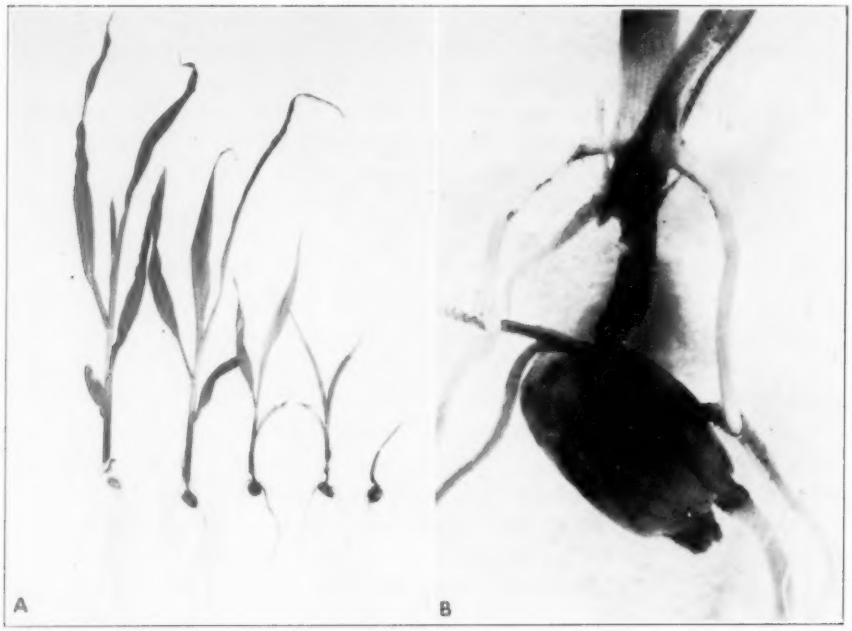

FIG. 1. *Sclerotium bataticola* on maize seedlings (inbred line Hy) after 3 weeks in infested steamed soil. A. A healthy seedling (on the left) and 4 diseased seedlings showing different degrees of stunting and disease development on the mesocotyls and primary roots. B. A close-up view of the second seedling from the right in A. Sclerotia were abundant on the pericarp of the seed, seminal roots, mesocotyl, coleoptile, and crown. The cortex and much of the epidermis of the seminal roots had disappeared.

the less affected tissues was not always indicative that the fungus was not present. The fungus always was isolated from necrotic tissues beyond the regions of sclerotia and occasionally from apparently healthy regions adjacent to necrotic areas without sclerotia. Sclerotia were observed extend-

TABLE 1.—*Disease development on maize seedlings grown in steamed soil infested with Sclerotium bataticola and held at 11.5° C. for different periods before being transferred to the greenhouse at 25° C.*

Soil treatment	Days at 11.5° C.	Seedling height[a]	Disease severity[b]	
			Mesocotyl	Roots
	No.	*cm.*		
Infested	0	32.2	58.4	60.7
	2	32.4	47.5	35.9
	4	38.8	32.1	37.1
	6	30.4	38.1	25.8
Non-infested	0	41.2	8.3	6.0
	2	34.6	7.4	6.2
	4	35.9	7.4	2.5
	6	37.0	4.8	1.2

[a] Mean of 4 replications.

[b] $\frac{\text{Sum of ratings of individual plants} \times 100}{\text{Number of plants examined} \times 3}$.

TABLE 2.—*Disease development on maize seedlings in Sclerotium bataticola-infested and noninfested steamed and nonsteamed soil with treated and nontreated seed*[a]

Seed treatment	Soil steaming	Soil infestation	Location of inoculum	Amount of inoculum	Seedling height[b]	Seedling weight	Disease severity	
							Mesocotyl	Roots
				gm.	*cm.*	*gm.*		
None	Steamed	None	.....	.....	35.7	27.8	13.6	9.9
"	"	*S. bataticola*	Seed level	5	36.4	26.6	100.0	71.5
"	"	"	"	10	37.0	25.1	96.5	64.3
"	"	"	"	20	32.6	21.3	94.1	69.1
"	"	"	Above seed level[d]	20	34.2	22.7	97.6	46.9
"	"	"	Below seed level[e]	20	30.0	23.7	96.3	66.7
"	"	*F. moniliforme*	Seed level	20	32.9	27.6	13.1	1.2
"	"	*F.m.* + *S.b.*[c]	"	20 (each)	32.4	22.6	77.4	22.6
"	Nonsteamed	None	.....	.....	35.5	24.8	15.5	4.8
"	"	*S. bataticola*	Seed level	20	33.2	20.0	9.0	1.3
Semesan Jr. (soak)	Steamed	None	.....	.....	37.8	33.3	3.6	0.0
"	"	*S. bataticola*	Seed level	20	34.4	26.8	84.6	48.8
"	"	"	Above seed level[d]	20	35.6	26.4	60.7	4.8
"	"	"	Below seed level[e]	20	35.6	26.8	83.4	45.3

[a] Greenhouse temperature approximately 25° C.
[b] Mean of 4 replications.
[c] *Fusarium moniliforme* and *Sclerotium bataticola*.
[d] 1½ cm. above.
[e] 2 cm. below.

ing along the seminal roots as much as 8 cm. from the seed. The development of necrosis began at the seed (location of the inoculum) and progressed for varying distances along one or all members of the primary root system and along the mesocotyl. The fine branch roots also became infected and eventually disjoined. Secondary roots became infected following complete necrosis of the mesocotyl during the early development of the seedling. An occasional lesion with sclerotia occurred on the secondary roots that passed down through the region where the inoculum was applied, but usually they were free of infection. The fungus also was isolated from the scutellum and from the plumular region of the more severely stunted seedlings.

The disease was more severe at 25° to 30° C., than at lower temperatures. Maintaining the seed in contact with inoculum in cold, wet (steamed) soil

TABLE 3.—*Disease development on 4 inbred lines and 2 single crosses of dent maize in steamed soil infested with Sclerotium bataticola*[a]

Maize seed	Soil infestation[b]	Seedling height[c]	Seedling weight	Disease severity	
				Mesocotyl	Roots
		cm.	*gm.*		
Inbred line 1	Infested	37.7	22.9	50.9	36.8
	Noninfested	40.4	32.1	4.8	1.6
2	Infested	32.1	23.0	63.3	26.7
	Noninfested	33.3	25.1	4.8	0.0
3	Infested	37.9	26.1	20.6	9.5
	Noninfested	39.6	36.8	3.2	1.6
4	Infested	37.8	22.8	70.0	36.7
	Noninfested	40.9	29.4	7.9	3.2
Single cross 1	Infested	38.6	31.9	79.7	48.2
	Noninfested	42.0	32.2	12.7	4.8
2	Infested	41.2	31.2	13.3	8.3
	Noninfested	44.9	38.7	6.4	3.2

[a] Greenhouse temperature approximately 25° C.
[b] 20 gm. inoculum placed at the seed level.
[c] Mean of 3 replications.

for 2–6 days before transferring to the greenhouse at 25° C. resulted in less disease under longer exposures to these conditions (Table 1).

Similar disease development occurred with applications of 5, 10 or 20 gm. of inoculum to each pot of soil or by placing inoculum 2 cm. below or 1½ cm. above the seed level (Table 2). Treating the seed by 15 minutes' soaking in a 0.001 per cent water solution of ethyl mercury phosphate only slightly reduced disease development. Less disease occurred from inocula of *Fusarium moniliforme* and *Sclerotium bataticola* mixed immediately before their application to the soil than from *S. bataticola* alone. No disease occurred when inoculum of *S. bataticola* was added to nonsteamed soil.

The activity of *S. bataticola* apparently was influenced considerably by the antibiotic activities of other soil organisms. The fungus was able, however, to grow through steamed soil, as disease occurred on the mesocotyl

when the inoculum was placed below the seed level and on the roots when placed above the seed level. The failure of seed treatment markedly to reduce disease suggests that avenues other than seed may be available for the entrance of the organism into the mesocotyl and primary roots. While diseased tissues on agar media frequently yielded *Fusarium moniliforme* and saprophytic organisms along with *Sclerotium bataticola,* such organisms were viewed only as of secondary importance.

Differences in disease development on several inbred lines and single crosses of dent maize were noted in one test (Table 3). These results suggest that there are differences in susceptibility to this disease and that the seedling reaction might be used in finding sources of resistance to it.

SUMMARY

A seedling disease of dent maize caused by *Sclerotium bataticola* Taub. is described as obtained in the greenhouse. Severe mesocotyl and primary root necrosis with accompanying stunting of seedlings was obtained only under steamed soil conditions where the suppression of *S. bataticola* by other soil organisms was reduced to a minimum. Differences amongst inbred lines and single crosses of dent maize seedlings in susceptibility to the disease were noted.

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PHYTOPATHOLOGICAL NOTES

The Use of Abrasives for Inoculating Sugar-cane Seedlings with the Mosaic Virus.—In the routine testing of sugar-cane varieties for disease resistance at the U. S. Sugar Plant Field Station at Houma, Louisiana, from 15,000 to 25,000 new seedlings are inoculated each year with the mosaic virus as the first step in the elimination of disease-susceptible ones. The Matz[1] method is used, in which the inoculum is placed in the space between the youngest expanded leaf blade and the next younger leaf that is still rolled, and a fine needle then passed 6 to 8 times through the liquid and into the basal portion of the unexpanded leaves (the spindle).

The method is usually 90 per cent or more effective with susceptible varieties, but for inoculating several thousand small plants, such as those grown from true seed, it is tedious and time-consuming. Furthermore, plants grown from true seed are too small for rapid inoculation by the needle-prick method until they are several weeks old. Fewer seedlings can be handled in a season because of not being able to move the first-inoculated, potted seedlings from the greenhouse earlier and replace them with new plants.

It was desirable to develop a method which would be equally or more effective, but faster and less tedious than the needle-prick method. The abrasion technique, devised by Rawlins and Tompkins,[2, 3] or a modification thereof, appeared suitable if a high enough percentage of infection would be obtained. A preliminary experiment was made according to the following procedure: In one series, finely ground, unwashed, white, sea-island sand of 100-mesh grade, to which inoculum was added, was picked up between the thumb and index finger and rubbed on the spindle. In another series, 100-mesh carborundum was used in the same way. Enough pressure was exerted by the fingers to rupture the tissues to the extent that they appeared to be water-soaked. No effort was made to proportion the amounts of inoculum and abrasives, but enough of the former was added to make a mixture of thin consistency. The needle-prick method was used as control. Two-month-old seedlings, 3 to 6 inches tall, of the cross Co. 281 × U.S. 1694 were used because, in the past, seedlings of this cross had shown a relatively high degree of susceptibility to the mosaic virus. There was injury to the young plants but, insofar as could be determined, this injury did not affect their normal growth to any appreciable extent. The results are presented in table 1.

Comparatively high percentages of infection were obtained with both sand and carborundum as abrasives, the percentage of plants in each in-

[1] Matz, Julius. Artificial transmission of sugar-cane mosaic. Jour. Agr. Res. [U.S.] 46: 821–839. 1933.

[2] Rawlins, T. E., and C. M. Tompkins. The use of carborundum as an abrasive in plant virus inoculations. Phytopath. 24: 1147. 1934.

[3] ———— and ————. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopath. 26: 578–587. 1936.

stance being more than 3 times as great as by the needle-prick method. In other tests, comparable infection was also obtained by rubbing only the last expanded leaf with sand and inoculum. It was recognized, however, that in using as test plants those from a seedling progeny that differed genetically in their susceptibility to mosaic, strictly comparative data for the methods could not be obtained.

The results were highly encouraging and warrant further testing of the abrasion method of inoculating young seedlings. If in further comparative trials the percentages of infection obtained approximate those obtainable with the needle-prick method when it is used with older plants, then the abrasion method may very advantageously replace the method now used. One man could inoculate by the abrasion method approximately as many plants in a day as four men could by the needle-prick method.

In other experiments with plants about two months old, which were grown from cuttings of a susceptible variety, the results were not consistent.

TABLE 1.—*Results of inoculating sugar-cane seedlings with mosaic virus by the abrasion and needle-prick methods. Readings made 4 weeks after inoculation*

Method of inoculation	No. plants inoculated	Plants infected	
		No.	Per cent
Sand	50	22	44
Carborundum	50	21	42
Needle-prick	100	13	13

Infection was obtained by the abrasion method in some tests but not in others. It appeared that maturity of tissue and possibly particle size and cutting qualities of the abrasives influenced the results, and that these factors must be considered in further developments of the method, particularly with plants grown from cuttings.—DOUGLAS C. BAIN, United States Sugar Plant Field Station, Houma, Louisiana.

A Method of Producing an Epiphytotic of Tomato Fruit Rot in the Field.[1]—Fruit rot of tomato (*Lycopersicum esculentum* Mill.), caused by *Phytophthora capsici* Leonian, has been responsible for severe losses in Colorado canning areas during seasons when weather conditions were favorable for the development of the disease.[2] To determine the effectiveness of certain fungicides in controlling the disease and to test certain tomato varieties and types for resistance, field plots were planted at Fort Collins in 1942 and 1943. Because of the probability that the field soil there might not contain a concentration of *Phytophthora capsici* sufficient to bring about an epiphytotic of tomato fruit rot, a method was devised to produce sufficient sporangia for large-scale field inoculations.

[1] Published with the approval of the Director as Paper No. 182, Scientific Journal Series, Colorado Agricultural Experiment Station. The writers wish to express their appreciation to E. W. Bodine, Associate Plant Pathologist, Colorado Agricultural Experiment Station, for his assistance in the preliminary phases of this study.

[2] Kreutzer, W. A., E. W. Bodine, and L. W. Durrell: Cucurbit diseases and rot of tomato fruit caused by *Phytophthora capsici*. Phytopath. 30: 972–976. 1940.

In preliminary laboratory trials isolates of the fungus obtained from naturally infected tomato fruits were grown on steamed corn meal, steamed barley, steamed potato cubes, steamed pepper fruits, oatmeal agar, potato-dextrose agar, in water culture with pieces of fresh green and red tomato fruits, pepper fruits and stems, and in 1 per cent peptone solution. Few or no sporangia were produced by the fungus during 40 days at 25° C. on the solid media. Although sporangia were produced in most of the water cultures they were not in sufficient quantity to supply the needed inoculum.

The method finally developed was as follows: After the fungus was grown on steamed barley in 250-ml. Erlenmeyer flasks for 20 to 60 days at 25° C., the contents of each flask were passed through a food grinder and thoroughly mixed into the upper 3 inches of unsteamed field soil in a wooden flat. The soil then was watered thoroughly and covered loosely with paper to prevent excessive moisture loss. Since earlier tests had shown that soil aeration was necessary for satisfactory sporangial production, the soil was turned with a trowel once or twice daily. At the end of 24 and 48 hours sporangia were abundant. In order to determine whether there were sufficient sporangia to produce adequate inoculum in the form of swarmspores, samples of approximately one cubic inch of the soil mixture were placed in 400-ml. beakers containing about 30 ml. of tap water. Care was taken not to cover the soil completely with the water. After 1 to 3 hours at 25° C., abundant swarmspores were in each drop of the water examined microscopically. Swarmspores so produced readily caused infection. When drops taken from one of the beakers were placed on the uninjured surfaces of either green or red tomato fruits which were then incubated in a moist chamber at 25° C., infection was observed within 24 to 48 hours. Isolations from infected areas invariably yielded cultures of *Phytophthora capsici*.

To obtain adequate material for field-soil inoculations in 1942, isolates of *Phytophthora capsici* were grown for 60 days on steamed barley in one-quart milk bottles. One hundred and ten of these cultures then were ground and mixed with approximately 1⅓ cubic yards of unsteamed soil in a large wooden frame adjacent to the test field. The inoculated soil was moistened with a fine spray and covered with paper. At 8- to 12-hour intervals the soil was shoveled over to insure adequate aeration. Water was added when necessary to maintain the moisture content of the soil-inoculum mixture. At daily intervals from the third to the eighth day following its initial preparation a shovelful of the soil-inoculum mixture was placed in each of several 4-gallon glazed earthenware jars containing approximately a gallon of ditch water. Drops of water taken from these jars were examined microscopically every 30 minutes for 3 hours. Abundant swarmspores were present in these random drops in from 1½ to 2½ hours after exposure at 75° to 85° F.

In inoculating the soil where the test plants were growing, the prepared soil-inoculum mixture was spread thinly at the bases of the plants during

irrigation and shovelfuls of the inoculum were also placed at the edges of the running water at 25-yard intervals in the irrigation furrows. The soil was irrigated by the furrow method at sufficient intervals for the next 10 days to keep the surface moist. In addition an overhead sprinkler was used for the first three days following the soil inoculation. More than an acre of soil in which approximately 2,500 tomato plants were growing was inoculated in 1942. In 1943, this same method was used to inoculate the soil in field plots in which approximately 600 plants were growing. An overhead sprinkling system was substituted for the furrow irrigation used the previous season. This large-scale method of inoculation resulted in uniform infection of tomato fruits over the entire field plots in 1942 and 1943.—W. A. KREUTZER AND L. R. BRYANT, Colorado Agricultural Experiment Station, Fort Collins, Colorado.

Tomato Seed Treatment with New Improved Ceresan Dust.[1]—Ethyl mercury phosphate has been used successfully by New Jersey seedsmen for treating seventy to eighty thousand pounds of tomato seed annually during the past six years. The seed is immersed for 5 minutes in a 1–1200 suspension of New Improved Ceresan (5 per cent ethyl mercury phosphate), allowed to drain for 25 minutes, then centrifuged and dried. Samples of treated seed plated on potato-dextrose agar are sufficiently free from surface-borne organisms to meet the rigid requirements of the Georgia Department of Entomology for producing Georgia certified plants. In addition the seed remains free from contaminating organisms for an extended period of time and is protected against seed decay and damping-off organisms, benefits which are not obtained from the mercuric chloride dip previously used.

Though both the material and the method now in use are excellent for disinfesting the seed, an equally effective treatment which is less laborious and less toxic to those who treat and handle the seed would be desirable. In an effort to find a substitute material, 37 fungicides, including some of the newer materials such as Arasan, Spergon and Fermate, were tested but none was found which gave seed protection and at the same time would meet the Georgia plant certification requirements. However, a simplified method using New Improved Ceresan as a dust shows definite promise. This method, in which a dosage of 0.5 per cent by weight is used and the seed agitated for 5 minutes, has consistently given 98 to 100 per cent clean seed in many laboratory plating tests.

No seed or seedling injury has been observed in any greenhouse experiments in which a 0.5 per cent dosage has been used. Furthermore, treated seed (0.5 per cent dosage) held for 12 weeks in stoppered test tubes which prevented the escape of the ethyl mercury phosphate fumes, showed no injury when planted in soil in the greenhouse. Slight seedling injury has been noted in pure sand at dosages of 1 per cent and higher, and in soil at 2 per cent and higher.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.

Plating tests show that the dust treatment prevents recontamination of seed held in cheesecloth bags exposed to air contaminants for as long as 28 weeks; and in all germination tests the dust method has been equally as effective as the dip method in preventing seed decay and damping-off.

Forty pounds of seed representing four lots have been treated in our laboratory and composite samples have met the standards required for production of Georgia certified plants. Of the four samples submitted to the Georgia Department of Entomology, two were 100 per cent clean, and the other two, 99 per cent. These lots of seed are being planted in Georgia in the spring of 1944 to test the relative effectiveness of the dust and dip methods.

The effectiveness of a commercial machine duster in applying New Improved Ceresan dust to 100 pounds of tomato seed has been tested. Nine 100-seed samples taken from three machine-dusted lots were plated and found to have only seven contaminated seed. Of these, five samples were 100 per cent clean, and no more than 2 per cent fungus- or 1 per cent bacterial-contaminated seed were found in any of the other four samples.

In all experiments so far the dust method of applying New Improved Ceresan to tomato seed has proved to be as effective as the dip method in surface-sterilizing the seed, preventing recontamination, and protecting the seed and seedling against decay and damping-off organisms. A dosage of 0.5 per cent by weight of seed, applied either by laboratory methods or by means of a commercial machine duster, has consistently produced 98 to 100 per cent clean seed with no apparent seed injury in any experiments. This promising method decreases the danger of chemical injury to workmen and eliminates the time-consuming and laborious dipping, centrifuging, and drying processes necessitated by the liquid method.—B. H. DAVIS and C. M. HAENSELER, N. J. Agricultural Experiment Station, New Brunswick, New Jersey.